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Bioassay of Ciguatera Toxin*

THE tropical fish poisoning known as 'ciguatera', which is clearly different from the well-documented tetraodon poisoning, has long been known in the Pacific. Its narrow regional appearance and its variability within a given species of marine fish have been puzzling phenomena. The symptoms associated with ciguatera have been well described, but fundamental knowledge of the toxin is virtually non-existent. The present state of this problem has recently been reviewed by Randall[1].

As the first stage of an investigation aimed at the isolation and identification of the toxic principle, it was necessary to develop a reliable bioassay. The bioassay which had been devised for *Gonyaulax* toxin from shellfish[2] was used and standardized for ciguatera toxin[2] by Halstead and Bunker[3]. A re-investigation of this method in this Laboratory proved that this assay could not be employed with confidence and emphasized the necessity of finding an unambiguous bio-

Table 1. COMPARISON OF BIO ASSAY METHODS

	Water extract (5 mice used for each specimen)	Intraperitoneal injection in mice					
		Diethyl ether extract, in mgm. extract/gm. mouse. Figures represent mean time to death in min. of two mice tested at each level					
		0·1	0·3	0·5	0·7	1·0	1·5
Specimen (*Lutjanus bohar*)							
Highly toxic							
1	*N.D.**	(348)†	189	130	63	—‡	—
2	*N.D.*	(1,627)	249	155	111	69	89
3	*N.D.*	*N.D.*	240	81	90	43	30
4	*N.D.*	(360)	90	44	63	62	43
5	*N.D.*	*N.D.*	261	139	78	72	49
6	*N.D.*	308	128	101	89	—	—
Moderately toxic							
7	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—
8	*N.D.*	*N.D.*	788	850	108	—	—
9	*N.D.*	*N.D.*	(2,800)	750	—	124	—
10	*N.D.*	*N.D.*	—	*N.D.*	—	(367)	—
11	*N.D.*	*N.D.*	*N.D.*	1,252	—	1,044	—
12	*N.D.*	*N.D.*	*N.D.*	(1,620)	—	—	—
Non-toxic							
13	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—	2,800	—
14	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—	*N.D.*	—
15	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*
16	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	1,000
17	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—
Pristipomoides microlepis (Control)	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*

* *N.D.* signifies no deaths in the 72-hr. test period.
† Figures in parenthesis are times to death in one mouse of pair tested, the other living through test period.
‡ Dash indicates no tests made at this level.

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assay. As is shown by the results in Table 1 aqueous extraction of toxic fish as described by Halstead and Bunker[3] failed to extract the toxin. It was found that fish or fish extract could be screened for toxicity by feeding raw fish or extract residue mixed with egg to the mongoose (*Herpestes mungo*). This animal exhibits symptoms not unlike those which have been described for humans following ciguatera poisoning. With this qualitative assay it could be shown that ethanol will extract efficiently the toxin from *Lutjanus bohar* (Forksål) collected in the Line Islands and known to be toxic as a result of mongoose feeding. The extraction residue after removal of the ethanol *in vacuo* was injected intraperitoneally into mice. While such an extract would kill mice, so would a similar extract of a non-toxic control fish *Pristipomoides microlepis* (Bleeker), although at a higher dose-level. Satisfactory differentiation of toxic and non-toxic fish could be achieved with a more highly purified extract. The initial ethanolic extract of dried fish flesh was concentrated *in vacuo* and diluted with thrice its own volume of water. The resulting solution was first extracted with light petroleum, then with ether. The bulk of the toxin was found in the ethereal extract. This extract after removal of the solvent when injected intraperitoneally into mice (closed colony Carworth Farms Webster strain, weighing between 19 and 21 gm.) would produce kills at a level of 0·2 mgm./gm. while the ethereal extract of the control fish caused no symptoms at a level of 1·5 mgm./gm. The results of these experiments are included in Table 1.

While sufficient amounts of toxic extract for injection of larger numbers of animals have not been available for a quantitative bioassay, the assay method described above allows reliable recognition of ciguatera toxin. Further research aimed at the isolation and identification of the pure toxin is in progress.

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Studies in the Helminthocladiaceae (Rhodophyta): *Helminthocladia*[1]

MAXWELL S. DOTY[2] AND ISABELLA A. ABBOTT[3]

DURING RECENT WORK in the field with the algae of Hawaii several interesting red algae have been found. Two of these interesting algae which seem to be members of the Helminthocladiaceae are reported here, in the hope that more work with such algae will be encouraged.

The Helminthocladiaceae is accepted as being a family, the limits of which would include eight genera that are rather well known and perhaps three genera that are not well known, *Ardissonea, Dorella,* and *Endosira. Ardissonea* was described by J. Agardh (1899: 99) and is treated by Kylin (1956: 127), under the name of *Neoardissonia* Kylin, as a member of the Naccariaceae. *Dorella,* which may be a member of this family, has terminal cortical cells which are not enlarged. According to a personal communication from Dr. H. B. S. Womersley, the type of *Ardissonea* is a very finely branched alga and *Endosira* appears to be a juvenile of a different order. Kylin (1956: 557) suggests that *Endosira* may be related to *Nemastoma.* Consequently, we shall consider these genera no further in connection with the algae being described below.

Of the eight easily recognizable genera, only *Helminthocladia* possesses lateral carpogonial branches and zygotes (post-fertilization carpogonia) which divide transversely, longitudinally, or obliquely and give rise to a dense gonimoblast from both division products. In addition, in *Helminthocladia* the terminal vegetative cells in the cortex are strongly enlarged. Kylin (1956: 108) uses this latter as a key characteristic to separate this genus from *Helminthora.* There are other differences between the two genera: In *Helminthora,* for example, only the upper cell of a transversely dividing zygote gives rise to gonimoblast filaments. *Trichogloea* differs from *Helminthocladia* and from other well-known genera in having straight terminal, rather than curved lateral, carpogonial branches and in its calcification. *Dermonema* has long been a relatively unknown genus but is distinct in form, being erect cushions formed of noncalcified closely dichotomous branches, sometimes like *Chnoospora minima* in looks and habitat. Both *Dermonema* and *Cumagloia* (Gardner, 1917: 401) are distinct in having a diffuse gonimoblast ramifying among the cortical filaments near the zygote from which it originated as a few protuberances with no previous division of the zygote. The genus *Liagoropsis* of Yamada (1944) is like *Nemalion* (Desikachary, 1957*a*), having straight carpogonial branches, but differs in being calcified.

On the basis of various characteristics the algae to be described are judged to be distinct, new species of *Helminthocladia.* They represent the only records of this genus of the Rhodophyta for the Central Pacific Ocean.

Helminthocladia simplex sp. nov.
Figs. 1–18

DESCRIPTIO TYPI: Thalli irregulariter cylindrici, usque ad 9.5 cm. alt., acibus ramisque subsimplicibus saepissime 1 ad 1.5 mm. diam. Rami pauci, irregulariter dispositi. Thalli saepe simplices, qui saepe latiores quam thalli ramosi, raro, autem, plus quam 2 ad 4 mm. diam. Thalli simpliciores forma magis irregulares, clore magis obscuri, statura breviores saepe carpogoniales sunt. Thalli antheridiales ubique vel

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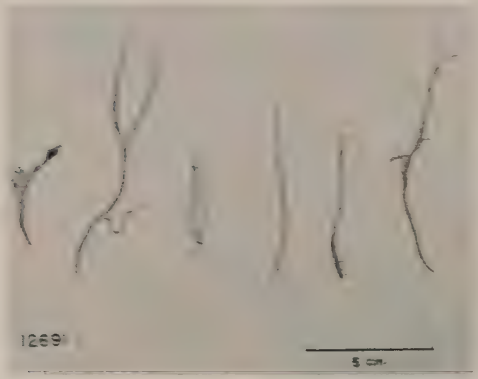


Fig. 1. The type of *Helminthocladia simplex*, a preparation on a herbarium sheet.

plerumque tenuiores, clore nitidiores, altiores, magis ramosi. Thalli textura ubique lubrici mollesque. Frondes multiaxiales, filamentis corticalibus nullo modo inter se adhaerentibus.

The holotype is a preparation of six thalli on one herbarium sheet deposited in the Bernice P. Bishop Museum in Honolulu, Hawaii. These specimens, along with a small *Liagora*, formed a turf on an almost horizontal rock surface just above extreme low tide line. The type material was collected by Jan Newhouse and Henry Kekoanui (M. Doty no. 12691) at Kahanahaiki, Waianae, Oahu, Hawaiian Islands (21° 32′ N., 158° 14′ W.), Jan. 2, 1954. Isotypes are being sent to the herbaria of the University of California, University of Michigan, Hopkins Marine Station of Stanford University, Cryptogamic Laboratory of the Paris Museum, University of Adelaide, South Australia, and Hokkaido University at Sapporo, Japan.

During some of the years since the original collection was made by Newhouse and Kekoanui, the type locality has been revisited. The sand shifts a great deal throughout the year at this site; sometimes the place is completely covered with sand. Until recently the alga had not been refound, though other members of the Helminthocladiaceae were often present in abundance. However, on Apr. 10, 1960, while Doty was accompanied by Newhouse and Ernani Menez, a dense stand of *H. simplex* (M. Doty no. 19135A, Menez no. 201) was found. The thalli were essentially of the same morphological form and were collected in the same place under the same conditions as the type. The living material was yellowish brown, with no taste or odor, and hard like a *Gracilaria* rather than soft like a *Trichogloea*. Perhaps a half liter of the species was obtained at this time. In time the algal population dwindled and the sand as well, until by May 8 there was very little of either on the site. The form of the *Helminthocladia simplex* present at this time was still the same as the other collections of this species, or perhaps a bit more eroded.

Thalli (Fig. 1) of irregularly cylindrical branches, up to 9.5 cm. tall, with most of the subsimple axes and branches 1–1.5 mm. in diameter. Branches irregular in arrangement and few. Thalli often simple, and these simple ones, while often of larger diameter than branched thalli, are rarely more than 2–4 mm. in diameter. Often the simpler thalli of more irregular form that are duller in color and shorter in stature are carpogonial. The antheridial thalli are generally more slender, more brightly colored, taller, and more branched. Texture rubbery and pliant throughout. The fronds are multiaxial and the cortical filaments do not adhere to each other in any way.

Our material is dioecious. No evidence of what might be a tetrasporangial generation was seen.

The male thalli produce spermatangia on terminal cortical cells among the vegetative filaments of the surface. The spermatangia are produced on cells (Fig. 2*s*) that are smaller than the adjacent vegetative cells and terminate in dichotomous rows of small cells. These small masses of cells do spread out under the cover glass on a microscope slide as do the terminal fans described for *Helminthocladia* by Martin (1939), but one suspects them of covering the surface area of the vegetative terminal cells they replace; i.e., occurring in round brushlike clusters rather than in two-dimensional fans. More than one spermatium may adhere to a trichogyne; those seen stuck to trichogynes were colorless.

The female apparatus develops laterally from the fourth or fifth cell (Figs. 3, 4, 5) below the enlarged terminal superficial cortical cell.

S
r
r
r
r
r
r
r
r
r
r
r
r
b
r1
r2
r3
r4
80 µ
50 MICRONS

The terminal cell of the developing carpogonial branch is large at first (Fig. 3). Only three-celled carpogonial branches were seen (Fig. 15 notwithstanding). It often appeared (Figs. 5, 6, 7) that the trichogyne cytoplasm became separated from the zygote cytoplasm; this we accepted as evidence that fertilization had taken place. Few cases were studied where we were certain that only the first division of the zygote had taken place. A number of cases were seen where two (Figs. 6, 7, 8), three (Figs. 9, 10, 11), four, or five divisions (Fig. 12) had taken place. From these it was clear that division of the zygote is usually longitudinal or oblique, as in the case of *H. papenfussii* as illustrated by Martin (1939, figs. 17, 18).

Conspicuous post-fertilization changes were not apparent in the carpogonial branch cells other than in those derived from the carpogonium itself. No placental cell formation was seen. Pit connections within the carpogonial branch (Figs. 13, 14) and to the supporting cell and to the supra-supporting cell were not enlarged, or those between the carpogonial branch cells were only slightly enlarged. The hypogynous carpogonial branch cells in older stages (Figs. 13, 14) were "lighter staining" than during earlier stages. In the six or eight cases in older gonimoblasts studied in this regard (e.g., Fig. 15), the central complex of densely staining cells presumably derived from the carpogonial branch had only, at most, "broadened" pit connections. The contents of the supporting and supra-supporting cell were in some cases darkened and shrunken in diameter in this formalin-fixed material.

As in *H. papenfussii* (Martin, 1939), *Helminthora lindaueri* (Desikachary, 1955: 131), and in *Helminthocladia australis* (Desikachary, 1957*b*), encircling sterile rhizoid-like filaments (*r* in Figs. 6, 7, 9, 11, 14, 15) after fertilization grow especially around the hypogynous cells of the carpogonial branch. These were not seen to invade or surround older gonimoblasts of *H. simplex* when these were producing surfaceward-growing filaments. In fact they seemed largely to have disappeared or become lost in our preparations of older stages.

Gonimoblast initials appear from both primary division products of the zygote (Figs. 9–13). The cells of the young gonimoblast (Figs. 10–12) are at first in a dense regular mass which becomes lobed (Figs. 13, 14, 15) in time. In this respect our organism is like other *Helminthocladia* species and unlike other genera in the family.

From the dense indefinitely lobed central gonimoblast mass, sparsely branched rather parallel filaments (Fig. 16) grow towards the surface of the thallus. The gonimoblast filaments are usually unbranched for the terminal three cells. It may well be, though not followed out closely, that the terminal two cells tend to become carposporangia and the bottom (third one) gives rise (Fig. 17*b*) to a two-celled branch that in turn grows to look like the terminal two cells of the parent filament before they began enlarging into carposporangia. This same third cell may produce another branch. It is interesting to note this sympodial manner of growth here.

In some cases the third cell in the row becomes a carposporangium. In this respect the organism at hand approaches that condition illustrated by Kylin (1930, fig. 2D) for *H. calvadosii*. That is to say, there is nothing like the branching which Papenfuss (1946, fig. 25) illustrates for the homologous structures in *Trichogloea*.

DISCUSSION: The type, MD 12691, is distinguishable from the classical *Helminthocladia hudsoni* and *H. calvadosii* (accepted as the type species of the genus as circumscribed by Hamel,

FIGS. 2–18. Reproductory and anatomical features of *Helminthocladia simplex*. 2, Cellular details of a cortical filament system bearing spermatangia (*s*) apically and a rhizoid (*r*) from the assimilatory region. 3–5, Young carpogonial branches (stippled). 6–15, Stages in the development of the gonimoblast with its enveloping rhizoids. Individual cells are indicated by dark stippling; groups of cells are indicated by light stippling; enveloping rhizoids are indicated, and an assimilatory apical cortical cell. 16–17, Branching of the carpospore-producing filaments (*b*). 18, Cellular details of a cortical vegetative filament system showing the strongly dichotomous branching and, from the inner cortical regions, rhizoidal filaments (*r*).

1930), and from all other species known to the authors, in the reproductory structures described for these taxa and on the basis of their being more branched and larger in size. Specimens of *H. calvadosii* from France (University of California Herbarium no. 407401, identified by Kylin) measured 26–36 cm. tall, and in the parts examined lacked any trace of the sterile rhizoid-like filaments characteristic of our species and of *H. papenfussii.* Another specimen (University of California Herbarium no. 218320, labeled by Rosenvinge *H. purpurea*) was up to 60 cm. tall and likewise lacked the peculiar rhizoidal filaments around the hypogynous cells. This latter specimen was the most nearly simple in branching of any *Helminthocladia* examined by us aside from *H. simplex.* In regard to the enveloping rhizoidal filaments our organism is unlike *H. calvadosii* (Kylin, 1930), *H. hudsoni* (Feldmann, 1939) which have no such filaments, and *H. papenfussii* as described by Martin (1939) which has many such filaments. The most striking of these sterile filaments (Figs. 8, 9, 12, 14) arise from the cell above the supporting cell in the vegetative branch, but they are more complex than those Balakrishnan (1955) illustrates for *Liagora erecta.*

Martin (1939) ascribes both a fusion cell to *Helminthocladia papenfussii,* derived from the carpogonial branch, and a sterile envelope; these are illustrated in her figures 20 and 21. By the time a gonimoblast is this far developed in this Hawaiian species, there is no indication of either such a fusion cell or such an enveloping basket of sterile branches. The sterile rhizoidal branches develop in *H. simplex* as in some other *Helminthocladia* species where such may be found, primarily from the cell above the supporting cell in the vegetative branch, as Kylin (1938, fig. 1C) illustrates *H. papenfussii.* The first to appear tend strongly to encircle the young gonimoblast but they were not seen in older stages. Desikachary (1956, figs. 25, 29) illustrates a similar situation in *Helminthora lindaueri* from New Zealand.

The material reported and figured as *Helminthocladia australis* by Okamura (1916: 21) and by Segawa (1957: 58, fig. 254) seems to be similar to ours in habit, except for the larger size and greater degree of branching. However, Narita's (1918) figure of *H. yendoana,* which in that author's opinion includes *H. australis* of Okamura, does not resemble our alga at all. Furthermore, our examination of certain specimens (University of California Herbarium nos. 335335, a female thallus apparently identified by S. Narita; 279932, a female thallus identified by Y. Yamada; and 418162) shows the Japanese material to be different in other details as well. From the materials illustrated and discussed as *H. australis* by Desikachary (1957*b*), our material differs in being far simpler and smaller.

We refer here only briefly to the rhizoids (Fig. 18*r*) which develop from the lower cells of the cortical filament systems. Only rarely was there any indication of such a rhizoidal filament (see *r* in Figs. 2) in the outer part of the cortex that even recalls slightly the rhizoids peculiar to the next species. Figure 18 r_1–r_4 perhaps illustrates the ontogeny of this rhizoidal type. Note that the cortical cells (Fig. 18) are, in general, characteristic for *Helminthocladia.*

Helminthocladia rhizoidea sp. nov.
Figs. 19–24

DESCRIPTO TYPI: Thallus 9 cm. alt., valde mucosoideus, in partibus inferioribus radiatim ramosus; filamenta corticea usque ad 350 μ long., irregulariter dichotome tritomeve ramosa; cellulae apicales amplificatae, pyriformes, 13–26.5 μ lat., 45 μ long.; rhizoidea multa 4.8–7.2 μ lat., e filamentis corticeis exterioribus producta; gonimoblastus involucro filamentorum e cellulis minoribus quam cellulae corticis vegetativi consistantium, aliter, autem, filamentis assimilativis corticeis propinquis similium, circumdatus; filamenta involucri e cellulis vegetativis infra superque cellulam sustinentem producta.

The holotype is a preparation bearing the collection number MDoty 12860. The specimen was collected by Mr. Tetsuo Matsui at Lahaina (156° 41′ W., 20° 53′ N.), on the island of Maui, Hawaii, and it is deposited in the Bernice P. Bishop Museum, Honolulu, Hawaii.

Thallus (Fig. 19) of cylindrical branches, 9 cm. high, branching radial in the lower portions, the branches 4 mm. in diameter at their bases, gradually tapering to 1–2 mm. in diameter at the tips, strongly mucosoid. The main branches give rise laterally to shorter ones of irregular length between 1 and 3 cm. long. Basal disc 5 mm. in diameter.

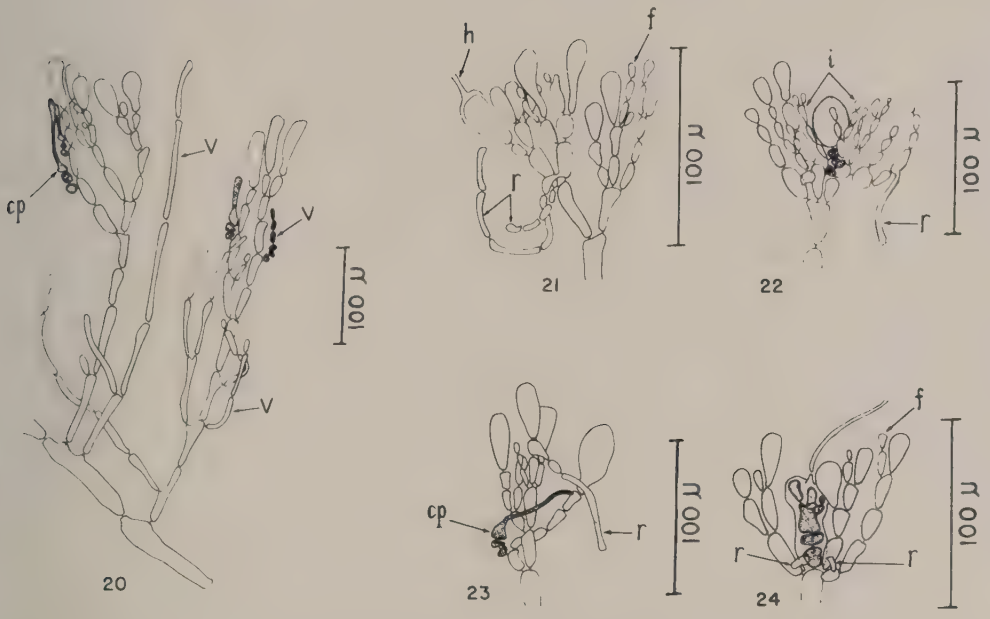
Cortical filaments (Fig. 20) up to 350 μ in length, irregularly dichotomous or trichotomous, the lower cells ovoid to cylindrical, the terminal cells inflated and pyriform, 13–26.5 μ wide $\times$ 45 μ long. Short unbranched filaments, usually without the terminal pyriform cells, are commonly borne at the tops of the cortical filaments (Figs. 21*f*, 24*f*). Numerous, sometimes branched rhizoids 7.2–12 μ wide are produced from the medullary filaments. Rhizoids are also produced (Fig. 21*r*) by upper cells of the cortical filaments at first as protuberances 4.8–7.2 μ wide on the lower edge of the cells (Fig. 22*r*), then by elongation cutting off segments (Fig. 23*r*). They are linear, unbranched, and seem to connect neighboring assimilatory branches to each other, whereas those rhizoids produced nearer the axial strand add thickness to the axis. Occasional hairs (Fig. 21*b*) may be formed on the terminal cortical cells.

FIG. 19. The type of *Helminthocladia rhizoidea*, a single specimen preserved on a herbarium sheet.

Carpogonial branches 3-celled, curved, mostly lateral as specially formed branches (stippled in Figs. 20, 23) at or near a dichotomy, but occasionally taking the place of a vegetative branch at a dichotomy. If unfertilized, they may develop into cortical filaments (Fig. 20) that are of smaller diameter than the ordinary cortical filaments. The first division of the gonimoblast is longitudinal. Gonimoblast dense, regular in shape (Fig. 22), soon becoming an irregularly shaped dense mass of filaments with only the end cells producing carpospores.

A few sterile filaments are produced from the vegetative cells above the supporting cell (Fig. 24) or from those subtending the supporting cell (Fig. 22). They loosely bracket and overtop the gonimoblast (Fig. 22*i*), together with the vegetative filaments deflected by the growth of the cystocarp. No fusion cell is formed.

DISCUSSION: In its vegetative appearance (Fig. 19), *H. rhizoidea* is similar to certain other species of *Helminthocladia,* such as some forms of *H. australis* (Desikachary, 1957*b*, pl. 16, fig. 3). It is a strikingly different alga from *H. simplex,* described above (Fig. 1), which it does not resemble in either external or internal structure. However it is generally similar in external appearance to other well-described species of *Helminthocladia;* i.e., *H. calvadosii* (Kylin, 1930), *H. papenfussii* (Martin, 1939), and *H. australis* (Levring, 1953; Desikachary, 1957*b*). *H. rhizoidea* differs from these because of the production of decumbent rhizoids (*r* in Figs. 21–23) from the basal ends of the vegetative cells, which constitute the assimilatory filaments. While this fact in itself may not be of first importance, it does clearly separate this species from other species of *Helminthocladia.* Rhizoidal structures do appear nearer the medulla in *H. simplex* (Fig. 2) and in the *Helminthocladia* studied by Desikachary (1957*b*: 442, fig. 5), but these seem to have a different origin (see Fig. 18). It would seem that vegetative



FIGS. 20–24. Cellular reproductive and vegetative peculiarities of *Helminthocladia rhizoidea*. 20, A cortical heterofilamentous system, wherein some branches are of slender long cells (*v*) and some terminated by "normal" obpyriform cells, normal (e.g., *cp*) and seemingly abnormal carpogonial branches. 21, Cellular details of a cortical vegetative system illustrating a hair base (*h*), slender cortical filaments (*f*), and two of the rhizoids (*r*) which characteristically issue from the assimilatory region. 22, Origin of a rhizoid (*r*) from an assimilatory cortical filament, a well-developed gonimoblast (lightly stippled) with three hypogynous cells (darkly stippled) and several small-celled involucral filaments (*i*). 23, One of the rhizoids (*r*) peculiar to this species and well-formed carpogonial branch (*cp*). 24, A young gonimoblast with two one-celled encircling rhizoids (*r*) developed from supra-supporting cells, and a slender cortical filament (*f*).

characters of this kind are necessary aids to distinguishing the ever growing number of species in this genus.

The sterile filaments surrounding the cystocarp of *H. rhizoidea* (Fig. 22*i*) appear to resemble closely those in *H. papenfussii* as illustrated by Martin (1939), although the derivation of the sterile filaments may not be the same in both species. Martin states that the sterile filaments arise from the vegetative cell above the supporting cell in *H. papenfussii*. This is true also in *H. australis* (Desikachary, 1957*b*) where, however, they may also arise from the cell below the supporting cell. The derivation in *H. rhizoidea* also may be from above or below the supporting cell.

The possession of a loose basket of sterile filaments around the gonimoblast in *H. rhizoidea* seems to furnish a further characteristic for distinguishing this species from *H. simplex*. Only the initial few cells of the involucre are illustrated in Figures 22 and 24 for *H. rhizoidea*, while perhaps the ultimate in development of rhizoids is given in Figures 6, 7, 9, 11, 14, 15 for *H. simplex*.

Most species of *Helminthocladia* appear to have fairly regularly dichotomous assimilatory filaments; see the illustrations of *H. calvadosii* (Kylin, 1930), *H. hudsoni* (Feldmann, 1939), *H. australis* (Desikachary, 1957*b*), and those of *H. simplex*, especially Fig. 18 in this paper. In this respect *H. papenfussii* and the present

species are similar to each other in that the branches near the tops of the filaments are often trichotomous. The ultimate cells of the cortical filaments are more crowded, therefore, than those of most other species. Often in this genus where the cortical filaments are not dichotomous the production of carpogonial branches or rhizoidal branches (of the type illustrated in Fig. 18) seems to have been involved. Either normal carpogonial branches may have appeared (Figs. 20*cp*, 23*cp*), or abortive carpogonial branches may have become reorganized (Fig. 20*v*), possibly into vegetative branches.

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A New Opisthobranch Mollusc from Hawaii

ALISON KAY[1]

THE OPISTHOBRANCH genus *Arthressa* was proposed by Evans (1950) on the basis of a revision of the genus *Volvatella* Pease 1860. Pease's genus was known only from four species, three described by Pease (1860, 1868), based on single specimens from the Pacific, and a fourth described by G. and H. Nevill (1869) from Ceylon. Evans (1950) pointed out several discrepancies in Pease's species descriptions, and, while retaining *V. fragilis* Pease, the type species, he proposed the genus *Arthressa* to include *A. cincta* (G. and H. Nevill) from Ceylon and *A. elioti* Evans which was newly described from Zanzibar.

Four specimens of a species congeneric but distinct from *A. cincta* and *A. elioti* have been collected in the Hawaiian Islands, and are here described as a new species.

GENUS *Arthressa* Evans 1950

SHELL: Thin, calcareous, covered by a thicker periostracum extending beyond the calcareous margin. Body whorl ovate, broadest in the middle, and contracting posteriorly to a spout. Anterior aperture as broad as shell in its anterior half. Right lip overlapping reflected left lip behind aperture; left lip depressed behind margin and passing at the junction of right and left lips under recurved left margin into a deep umbilical cleft; margin continuous with the spout. On left side of spout columella of a sunken spire of approximately three turns visible through periostracum.

ANIMAL: Tentaculate area of head rimless, frontal, and oriented ventrally. Dorsal tentacles short and smooth. Lateroventral tentacles deeply grooved laterally, slightly bilobed at tip. Eyes immediately posterior to grooves. Anal opening on dorsal surface of visceral mass beneath mantle. Foot small and triangular with blunt corners.

TYPE SPECIES: *A. cincta* (G. and H. Nevill). Ceylon.

Arthressa evansi, new species
Fig. 1

HOLOTYPE: Bishop Museum, no. 8901. 11 mm. in length; 6 mm. in breadth; collected in a tidepool, Diamond Head Beach Park, Oahu, Hawaii, November, 1956. Paratype: Bishop Museum, no. 8902. 11 mm. in length; 5 mm. in breadth; collected with holotype.

Length of anterior aperture more than half that of shell. Body whorl rounded, not flattened on the left, and with the spiral curve of the shell continued into it. Length of spout less than half shell breadth. Opening of spout triangular with a ventral slit continuous with aperture. Mantle edge smooth in spout. Animal orange, freckled with a darker shade, and with a band of freckling crossing body whorl. Foot similar in coloring to that of body but freckling of a lighter shade. Foot bluntly rounded, not extending anterior to head, and with a groove separating anterior two-thirds from posterior third. Animals extruded a viscid white substance from both posterior spout and anterior aperture. Specimens collected vary from 8–11 mm. in length and from 4–6 mm. in breadth.

The species has been named for the late J. T. Evans.

The specimens were collected in a tidepool on the reef flat of Diamond Head Beach Park, Oahu. They appeared to be associated with the algae *Padina* and *Gracilaria.* The mode of progression on a hard substrate is such that the anterior end of the foot extends forward and attaches to the substrate while the posterior portions move up. The animals readily suspend themselves upside down on the surface of the water, progressing as waves of muscular contraction pass across the foot.

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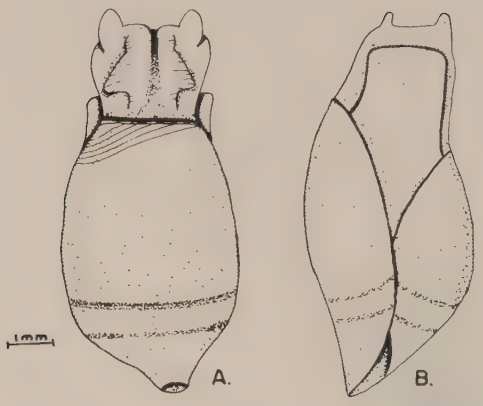

FIG. 1. *Arthressa evansi.* Drawn from the living animal. *A,* Dorsal view; *B,* ventral view.

DISCUSSION

Of the three species now included in *Arthressa, A. evansi* appears to be intermediate in characters between *A. cincta* and *A. elioti. A. evansi* resembles *A. cincta* in coloring and in the smooth mantle edge; it resembles *A. elioti* in the length of the anterior aperture and in the rounded body whorl.

Although Evans (1950) suggested that *Volvatella fragilis* Pease should be retained as it was sufficiently described, no collections of this species have been recorded in Hawaii since the original description. Pease (1860) merely described the type specimen as from the "Sandwich Islands," and the animal was illustrated as being white. Evans (1950) distinguished *Volvatella* from *Arthressa* on the basis of Pease's (1860) description of the anal opening into the posterior spout in *Volvatella;* the anus opens on the dorsal surface of the visceral mass in *Arthressa.* If specimens agreeing with Pease's (1860) description of *Volvatella,* including a posterior anus, are collected in the future, Evans' distinction between the genera will merit recognition; however, it is possible that Pease's description was misleading, and the pore which he described as the anus may have been the pore of the gland which secretes the viscid fluid which these animals emit. If this is the case, Evans could have gone further and entirely suppressed the genus *Volvatella* of Pease.

Evans (1950) described *Arthressa* as a saccoglossan genus, and it should therefore be removed from the position assigned *Volvatella* in the Akeratidae of Thiele (1931). *Arthressa* is of interest in the scheme of opisthobranch relationships in that it is a suctorial form feeding on various algae. The occurrence of a shell and a generalized mantle cavity and alimentary canal suggest that *Arthressa* holds a position in the Saccoglossa analogous to that of *Actaeon* (Fretter and Graham, 1954), which also possesses many prosobranchiate characters.

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CONTINENTAL MIGRANTS AND SHORE BIRDS ON OAHU DURING 1958/59

By Miklos D. F. Udvardy [1/] [2/]

During the fall and winter season of 1958/59 I spent 23 days on field observations of shore and water birds on Oahu. This field work resulted in the discovery of some rare migrant, or vagrant birds of continental origin. These data, and those rarities observed and published by others in the Elepaio, [3/] seem to indicate that many or most migratory water and shore birds of Pacific North America stray yearly in small numbers to the Hawaiian Islands, even if their presence is rarely recorded.

Besides these rarities, we have a numerical picture about the migratory shore birds and resident marsh birds that aids in assessing their ecological and zoogeographical status. In the accompanying Table I have included the records of the Audubon Society bird walks as well, in order to complete the seasonal picture of the principal marsh areas. All these localities are found on maps of 1:250,000 or larger scale.

Notes on the species:

Pintail (Anas acuta L.) Compared with Medeiros' six winter censuses of the early 1950-es, the estimated maximum number seen on the five major marsh areas, 575 birds, means a year of low pintail population on Oahu. The percentage of males in nuptial plumage, as calculated from my field notes, is the following:

Date	Number counted	% nuptial males
Oct. 7	39	0.0
Oct. 21	30	13.4
Oct. 28	80	13.8
Oct. 30	60	16.7
Nov. 4	93	22.5
Nov. 20	184	54.0
Dec. 4	170	41.0
Dec. 21	45	62.0
Jan. 8	358	48.0

Plotting these data on a graph, we can surmise that the sex ratio of pintails is slightly in favor of males; that about ten per cent of the males appeared in nuptial plumage before Oct. 21, and the main molting time of their majority was between Nov. 10 and Dec. 10, by which time about 50% of the pintail were nuptial males.

1/ Contribution No. 145 of the Hawaii Marine Laboratory.

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3/ Fulmar, Semipalmated plover, Snowy plover, Black-bellied plover, Glaucous gull, Herring gull, Least tern. See Dunmire 1959; Hanson 1958B, 1959; King 1958A, B, 1959A; Riggs 1959.

A tentative interpretation of these findings would be that after the time of arrival in Hawaii, the adult males molt first in the beginning of October and the juvenile males molt in the latter half of November and early December. If this assumption would stand, then we could conclude that about 20 or 25% of the wintering pintails are adult birds that might have learned their route in the previous year or years. They might have led the young of the year to these Mid-Pacific wintering grounds. The only proof of such a conclusion so far is the fact that Hawaii-banded pintails returned to the islands one to four years after their banding (Medeiros 1958).

Shoveller (Spatula clypeata L.) The bulk of about 130 shovellers censused on Oahu spent the winter on the east side of Kaelepulu marsh. The only sex ratio taken (Jan. 8, 1959) shows roughly even distribution of the sexes (48% males).

Pacific golden plover (Pluvialis dominica fulva/Gm./) and Ruddy turnstone (Arenaria i. interpres /L./) It looks as if we had a number of plover passing through Oahu during the late fall, or else the marsh concentrations at that time dissolved into smaller bands that occupy the fields and meadows, since there was a peak in their numbers around Nov. 20, 1958 according to the combined census date of Kahuku and Kaelepulu.

Date	Plover	Turnstone
End of Oct.	320	260
Mid-November	500	336
Late November	255	100
Early December	30	50

Canada goose (Branta canadensis) On my visit to Kaelepulu I found an individual of a small Canada goose among the pintails, on October 30, 1958. I did not see it during my two previous October trips, so it is reasonable to assume that it arrived between Oct. 28 and 30 to the marsh. Subsequently it has been seen there on Dec. 11, 1958 and Jan. 8, 1959 by myself, and on Jan. 25 and Feb. 23, 1959 by others. April 30, 1959 I did not see it any more on Kaelepulu. Most likely it left the marsh between Feb. 23 and March 25, since on that date Medeiros (1959) noted a cackling goose on the Kanaha pond of the island of Maui, about 90 miles East of SE. from Kaelepulu. I find it highly probable that this was the same bird that wintered on Oahu. This is the ninth noted occurrence of the species on the islands, and the second for Oahu.

(Lesser) Snow goose (Chen h. hyperbores /Pall./) Found October 21, 1958 at the mouth of Anahulu river, on the fish pond east of the bridge of the same river, in Haleiwa village, Waialua Bay, Oahu. When positively identified this bird was in the company of domestic (Pekinese) ducks, two pintails and an American widgeon. The pond further was populated by four moorhens and four-five coots. The juvenile snow goose was surprisingly tame and could be approached to within 40-50 feet when on the water or loafing with the white domestic ducks. I observed its presence on Oct. 21, 23, Nov. 4, 1958 and January 6, 1959. Hanson (1958B) also saw it Oct. 26, 1958, while MacClellan (1959) no longer found it on Jan. 25, 1959. The owners of the pond told me that the goose arrived about October 18, 1958 and left on or about January 20, 1959 without, to their knowledge, being molested. The only previous record of the snow goose on Oahu was in 1904, and altogether it occurred four times on the islands.

Green-winged teal (Anas crecca carolinensis Gm.) I have observed this species three times, but it is likely that the same group of four ducks occurred at Kahuku

on Nov. 20 and on Dec. 4, 1958, while on Nov. 27 I only could find one teal on the Kaelepulu marsh. The party of four was composed of two pairs, the males partially molted on November 20. This was the eighth noted occurrence in Hawaii, and the third for Oahu.

American widgeon (mareca americana /Gm./) Observed between Oct. 26, 1958 and Jan. 25, 1959 on all major marsh areas of Oahu, with its numbers varying from one to 20 at a time. It has been a regular wintering bird on Oahu since 1952, but its numbers are always very small.

Bristle-thighed curlew (Numenius tahitiensis /Gm./) Mr. G. Pearsall (verbal communication) saw two curlews at Kahuku on Oct. 18, 1958 and I was able to observe them at the same locality on Oct. 21, but they were no longer there on Oct. 26, 1958 (Hanson 1958B). Curlews, singles and couples are not seen every year on the main Hawaiian Islands.

Greater yellowlegs (Totanus melanoleucus /Gm./) I saw one bird at Kahuku on Nov. 4, 1958. This is the fifth noted occurrence on the islands, fourth for Oahu.

Western sandpiper (Ereunetes mauri Cabanis) I saw one specimen at Kahuku on Oct. 21, 1958. Presumably the same bird was seen there by Hanson (1958B) on Oct.26, but on Nov. 4, 1958 I no longer found it there. This bird was the first recorded from the Hawaiian Islands.

Summary:

Detailed observations on migratory birds are summarized. Besides seasonal fluctuations of the common species attention has been drawn to the fact, that eleven rare species were observed (five by the author) during a single season.

Acknowledgements:

The field work of this study was aided by a research grant of the National Research Council of Canada. Equally important was the support of the Hawaii Marine Laboratory that provided the transportation into the field.

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TABLE I: MARSH AND SHORE BIRD CENSUSES ON OAHU, 1958-59

DATE 1958/59	Black-crowned Night Heron	Pintail	Green-winged Teal	Am. Widgeon	Shoveller	Golden Plover	Ruddy Turnstone	Wandering Tattler	Sanderling	Hawaiian Stilt	LOCALITY OF OBSERVATION	REFERENCE
Jun 8	.	.	.	.	.	+	+	+	.	.	Moku Manu Is.	Hanson, 1958A
Aug 3	.	.	.	.	.	100+	20+	.	.	6-8+	Kaelepulu	Pyle, 1958
Aug 10	.	.	.	.	.	.	.	.	.	.	Salt Lake	Smail, 1958
Aug 23	.	.	.	.	.	1	5	1	.	.	Popoia Is.	King, 1958B
Oct 2	1	.	.	.	.	6	8	5	12	28	Kuliouou	*
Oct 7	.	39	.	.	.	(160)	23	.	10	34	Kaelepulu	*
Oct 21	.	120	.	.	2	(120)	(180)	4	.	129	Kahuku	*
Oct 26	.	+	.	+	+	+	+	.	+	+	Kahuku	Hanson, 1958B
Oct 28	.	155	.	1	3	+	+	.	.	.	Kaelepulu	*
Oct 30	.	60	.	13	14	(200)	(80)	.	.	3	Kaelepulu	*
Nov 4	.	93	.	.	1	(80)	(30)	.	6	15	Kahuku	*
Nov 4	.	50	.	.	.	.	.	.	.	.	Salt Lake	*
Nov 9	1	+	.	.	+	.	.	1	.	.	West Loch	Hanson, 1958B
Nov 9	.	+	.	.	.	.	.	.	.	.	Salt Lake	Hanson, 1958B
Nov 15	.	21+	.	.	.	.	.	2	.	+	Salt Lake	*
Nov 20	.	40	4	.	5	(300)	330	.	69	31	Kahuku	*
Nov 20	.	(350)	.	20	100	(200)	6	.	.	(60)	Kaelepulu	*
Nov 27	.	61	.	.	.	55	50	1	12	.	Kahuku	*
Nov 27	1	(350)	1	.	40	(200)	(50)	.	.	.	Kaelepulu	*
Dec 4	1	310	4	4	14	30	50	.	28	11	Kahuku	*
Dec 9	.	8	.	.	.	.	.	.	.	.	Salt Lake	*
Dec 9	5	5	.	.	.	2	.	1	.	.	West Loch	
Dec 11	.	(250)	.	4	110	.	.	.	.	(60)	Kaelepulu	*
Dec 21	2	(350)	.	.	42	44	35	.	2	58	Kaelepulu	Hatch, 1959
Dec 21	1	30+	.	2	+	15	18	5	2	35	Kuliouou + Kuapa	Hatch, 1959
Dec 21	1	.	.	.	.	78	31	3	.	27	Kaneohe	Hatch, 1959
Dec 21	.	58	.	10	.	6	.	.	.	4	Salt Lake	* (also Rockafellow, 1959)
Jan 8	.	358+	.	3	60	(30)	1+	.	.	(30)	Kaelepulu	*
Jan 25	1	+	.	+	+	+	.	.	.	+	Kaelepulu	MacClellan, 1959
Jan 25	.	+	.	.	.	+	+	.	+	+	Kahuku	MacClellan, 1959
Feb 20	.	.	.	.	.	40	30	.	.	.	Kahuku	*
Feb 23	.	+	.	.	+	+	+	.	.	+	Kaelepulu	Hanson, 1959
Mar 22	.	.	.	.	.	.	.	.	.	.	Salt Lake	Riggs, 1959
Mar 22	1	.	.	.	.	+	+	1	.	+	West Loch	Riggs, 1959
Apr 21	9	.	.	.	.	(60)	(20)	4	1	(40)	Kahuku	*
Apr 30	.	.	.	.	.	.	.	.	.	.	Kaelepulu	*
May 1	2	1	.	.	.	3	(80)	2	1	(40)	Kahuku	*
May 10	1	.	.	.	.	2	.	.	1	.	East Loch	King, 1959B
May 23	.	.	.	.	.	1	.	.	.	.	West Loch	*

Symbols: (60) - estimated number
+ - present, not censused
20+ - minimum number
* - observed by the present author

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THE OCCURRENCE OF THE MOCKINGBIRD ON THE ISLAND OF MAUI

By Miklos D. F. Udvardy [1]

During March 7-9, and again March 21-25, 1959, I had an opportunity to visit the island of Maui. I found the mockingbird (Mimus polyglottos /L./) a very common resident, with pairs occurring on every 4-5 acres of kiawe forest, in all the dry, kiawe-covered habitats that I visited, namely: the south-west foot of Mt.Haleakala, from Kakena, near sea level, to Ulupalakua, about 2000 ft. elevation; from there, along the highway toward Pulehu, between 2000-3000 ft.; also in West Maui, near Papawai Point, in the kiawe along the highway, near sea level.

From these observations I have concluded that the mockingbird is a well established member of the Maui avifauna, and it is abundant where suitable habitat occurs. A correction of the "Checklist and Summary of Hawaiian Birds" (E.H.Bryan, Jr., 1958), listing the mocking bird as abundant, regular breeder on Maui, would be appropriate.

While such a comprehensive picture of its abundance has not yet been noted, there were several indications, published in this journal, of its occurrence in the suitable areas of Maui, all between 1958 and 1960. G. C. Munro (1944, Birds of Hawaii) saw it in 1936, eight years after its introduction. H. S. Baldwin (Elepaio 11:69) and H. C. Bonsey (Ibid. 12:31) both noted its occurrence in 1951, but these are all the early records.

A desirable task for field observers going to Maui would be to assess the mockingbird population. Variation of its density (by censusing singing males in the morning) in the different kiawe-covered areas of the island, and mapping of these areas, would indicate the size of the breeding population. This would help in understanding the fact that this bird has overcome the 30-mile wide sea channel between Maui and Hawaii in, or shortly before, 1960 (C. Hanson, Elepaio 20:87).

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Contribution No. 146 of the Hawaii Marine Laboratory.

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Editor's Note: Any definite records of the possible introduction or transfer of mockingbirds to Maui would be welcomed as present records are inadequate.

Studies on Spawning Behavior, Egg Masses, and Larval Development in the Gastropod Genus *Conus*, Part I Observations on Nine Species in Hawaii

ALAN J. KOHN

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Studies on Spawning Behavior, Egg Masses, and Larval Development in the Gastropod Genus *Conus*, Part I Observations on Nine Species in Hawaii[1]

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THIS REPORT deals chiefly with the characteristics of egg masses and the course of larval development of gastropod mollusks of the genus *Conus*, based on material collected in the Hawaiian Islands during 1954–56, while the author was at the Hawaii Marine Laboratory. Some information has been obtained on 9 of the 33 species of *Conus* known to occur in the Hawaiian archipelago (Kohn, 1959*a*).

Bergh stated (1895: 100), "Ueber Paarung, Eilegen und Laich der Coniden ist...absolut nichts bekannt."

The only reference prior to that date which has come to the attention of the present writer is an extremely brief description of the oöthecae or egg capsules of *C. capitaneus* Linnaeus by Adams and Adams (1853: 5). Cooke (1895) and Hornell (1922) figured egg capsules of unidentified species of *Conus*, but Lamy (1928), in an extensive study of prosobranch egg capsules, noted their similarity to those of other genera. These early figures cannot be assigned with certainty to *Conus*, and Bergh's statement remained essentially correct until Petit and Risbec (1929) and Risbec (1931, 1932) described the egg masses and development of four species of *Conus* from New Caledonia. Subsequently, similar information on six additional species has been reported by Thorson (1940), Persian Gulf; Lebour (1945), Bermuda; Knudsen (1950), West Africa; Natarajan (1957), India. More recently Lewis (1960) has provided additional information on the egg capsules and larvae of *C. mus* Hwass *in* Bruguière, a species discussed also by Lebour (1945).

Ostergaard (1950) described the spawning and development of six species of *Conus* in Hawaii. The present paper provides information on five additional species and notes on development in one species mentioned by Ostergaard but not studied in detail by him.

Most of the material was collected on coral reefs which fringe much of the Hawaiian coastline and provide suitable spawning sites for a number of species of *Conus* (Kohn, 1959*b*). Bouin's solution and 10 per cent sea water formalin were used as fixatives. Except where stated otherwise, all material was collected by the author, and the numbers used to identify specimens are those of the author. The preserved egg capsules and embryological material have been deposited in the University Zoological Museum, Copenhagen.

GENERAL CHARACTERISTICS OF SPAWNING AND EGG MASSES

As is typical of the higher prosobranch gastropods, the sexes are separate and fertilization is internal. The prominent penis of the male is a flattened muscular tube which arises from the right side of the dorsolateral body wall just behind the head and is rounded or pointed at the tip (Bergh, 1895: 97, figs. 4, 5, 76, 149, 159).

In copulation, it is inserted into the vagina of the female, which is located anteriorly at the extreme right edge of the mantle cavity, where the mantle joins the body wall, and adjacent to the anus (unpublished observations). A detailed account of the genital apparatus of prosobranchs has been given by Fretter (1941, 1946).

Although the process of oviposition in *Conus* has not been studied closely, it is essentially similar to that first described in other higher prosobranchs (Order Neogastropoda) by Cunningham (1899) and later amplified by Ankel (1929), MacGinitie (1931), and Ino (1950). Eggs surrounded by albuminous, probably nu-

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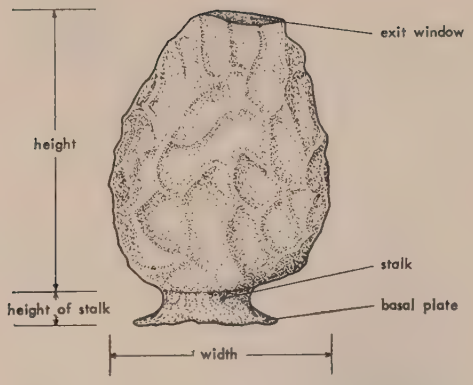


FIG. 1. Generalized egg capsule of *Conus*, with terms used in presentation of dimensions.

tritive, material are passed out of the genital aperture of the female downward through a temporary groove on the side of the foot to the egg capsule gland, which is situated on the anterior portion of the sole. The fluid mass of eggs passes into the aperture of the gland, in which horny egg capsules are produced. This method of egg capsule formation is characteristic of the Neogastropoda, in contrast to the Mesogastropoda in which the capsule is secreted in the genital ducts and only moulded and hardened by the egg capsule gland (Ankel, 1929; Fretter, 1941).

The general shape of the capsules (see Fig. 1) is "like a flattened pouch or flask" (Knudsen, 1950). As will be shown below and in a forthcoming paper (Kohn, MS), there is a moderate degree of interspecific variation in the form of the egg capsules and, in some species, considerable intraspecific variation. It is usually not possible to determine the species to which an egg mass belongs in the absence of the parent. This is in marked contrast to the egg capsules of some other prosobranch genera, e.g., *Murex* and *Nassa*, which are highly species-specific (Thorson, 1959).

The capsule is typically white or straw-colored and is presumably constructed of conchyolin, a scleroprotein. The capsules are deposited in clusters which frequently consist of several short rows of a few capsules each. A cluster of egg capsules is referred to as an egg mass, following Thorson (1940, *et seq.*) and others. The number of capsules deposited in a cluster and the number of eggs per capsule are variable. In the material collected in Hawaii, the number of capsules per cluster ranged from 1 to 68 (Table 1) and the number of eggs per capsule from 80 to about 10,000 (Table 2).

In most species, each capsule is attached to the substratum, usually the underside of a rock, by an adhesive basal plate (Fig. 1). There is a narrow, short stalk, above which is the capsule proper. Its walls are thin, nonrigid, and translucent, and they usually bear ridges on the flat surfaces. The lateral edges are typically convex, and there is a preformed exit window along the straight, uppermost part of the capsule. It is covered by a hyaline membrane or operculum, which is never very thick and appears to become thinner and more transparent as development proceeds. The hatching process has not been studied closely, but it appears that the entire membrane dissolves prior to escape of the larvae. This is in contrast to the family Muricidae, in which the egg capsule is closed by a thick operculum which is lost intact at hatching (Ankel, 1937; Hancock, 1956). Dissolution of the operculum may be due to an enzyme liberated by the larvae just prior to hatching (Ankel, 1937). The larvae of *Conus* do not occupy the entire capsule before hatching.

DEVELOPMENT WITHIN EGG CAPSULE

To study the course of larval development, egg masses were kept in constantly aerated running sea water in finger bowls or wide-mouth 4-l. jars. Following hatching, the larvae were kept in the larger jars in aerated, but not circulating, sea water. In some cases, sand and coral rocks from the normal habitat were placed on the bottom of the vessel in an effort to induce settling of the larvae, but this was generally unsuccessful. Details of embryonic development are described below for those species in which they could be determined, and present knowledge of development in *Conus* is reviewed in the section, Discussion.

Conus abbreviatus Reeve

A cluster of 33 egg capsules was found attached to the underside of a metal refuse can

TABLE 1

COLLECTION RECORDS AND CHARACTERISTICS OF EGG MASSES OF *Conus* IN HAWAII

SPECIES	LOCALITY[1]	DATE OF COLLECTION	SPECIMEN NUMBER	NO. OF CAPSULES IN CLUSTER	HEIGHT × MAXIMUM BREADTH OF CAPSULES (mm.)
abbreviatus	Waikiki	15 III 1923	(Ostergaard, 1950)	12	10 × 8
abbreviatus	Diamond Head	10 III 1956	1382	33	9 × 7–7.5
catus	Waikiki	12 VI 1922	(Ostergaard, 1950)	19	12 × 9
catus	Paia, Maui	5 VIII 1956	2180	1	12 × 10
catus	Koko Head	22 VIII 1956	2299	3[3]	11–12 × 10
catus	Koko Head	22 VIII 1956	——	4	10–11 × 8.5–9.5
ebraeus	Waikiki	22 III 1921	(Ostergaard, 1950)	—	10 × 10
imperialis	Diamond Head	6 VII 1956	1983	6[3]	18–20 × 12–13
leopardus	Kaneohe Bay	25 IV 1956	——	19	49–58 × 34–37
lividus (?)	Maile	7 II 1956	1264	36, 30	10.5–12 × 10–12; 9–10 × 8.5–10
pennaceus[4]	Waikiki	19 V 1921	(Ostergaard, 1950)	34	11 × 8
pennaceus[4]	Waikiki	25 V 1922	(Ostergaard, 1950)	—	——
pennaceus	Waikiki	26 VII 1954	156	20	8.5 × 7.5
pennaceus	Waikiki	4 VIII 1955	——	38	——
pennaceus	Diamond Head	13 VIII 1955	401–404	68, 50, 25, 9	——
pennaceus	Hauula	9 VI 1956	1811	34	9–10.5 × 7–8
pennaceus	Mahie Point	9 VI 1956	1810	58	9.5–11 × 7.5–9
pennaceus	Diamond Head	10 VI 1956	——	68	12–13 × 9–10
pennaceus	Diamond Head	4 VII 1956	1962	10	9–11 × 8
quercinus	Kaneohe Bay	9 II 1956	——	40	19–26 × 17–22
quercinus	Kaneohe Bay	9 II 1956	——	3	17–19 × 18–20
rattus	Waikiki	4 VIII 1921	(Ostergaard, 1950)	22	15 × 11
rattus (?)	Waikiki	21 III 1956	——	31	12–14 × 11–14
vexillum[5]	Waikiki	16 V 1921	(Ostergaard, 1950)	34	20 × 13
vitulinus	Ala Moana	7 VII 1956	2020	11	23 × 16–17
vitulinus	Diamond Head	VII 1957	——	—	——

[1] All from the island of Oahu unless otherwise indicated.
[2] Oviposition possibly interrupted by collector.
[3] Oviposition definitely interrupted by collector.
[4] This species is referred to as *C. omaria* Hwass by Ostergaard (1950).
[5] This species is referred to as *C. sumatrensis* Hwass by Ostergaard (1950).

cover in 1 m. of water on the reef at Diamond Head, Oahu, 10 March 1956. The capsules (Fig. 2) were affixed to their substratum in three rows of 12, 17, and 14. Two adult *C. abbreviatus* (no. 1382, ♀, 29 × 21 mm.; no. 1383, sex undet., 32 × 21 mm.) were found about 1 m. from the can lid, which appeared to be the nearest available protected site for oviposition, since all nearby coral rocks were buried to a depth below the oxidized surface layer of sand. This egg mass is tentatively assigned to *C. abbreviatus* because of the proximity of the adults and the close similarity with the egg capsules of that species described by Ostergaard (1950).

The shape of the capsules is typical of the genus. They are slightly higher than broad and are affixed to the substratum by a short stalk and broad basal plate. The capsular walls are smooth except for peripheral ridges on one side (Fig. 2*b*) and two ridges extending downward from near the upper corners on the opposite side (Fig. 2*c*). Both lateral edges of the capsule are convex. The characteristics of the egg mass are summarized and compared with that studied by Ostergaard (1950) in Tables 1 and 2.

In order to study the course of development, the egg mass was placed in aerated circulating sea water. One egg capsule was removed from the cluster and its contents examined on the day of collection. The embryos, which were pink in color when viewed through the translucent capsular wall, were found to be in the blastula

stage, with the four macromeres and many micromeres distinctly visible. It is likely that the embryos were 1–2 days old. They were oval in shape, measured 175–200 × 165–175 μ, and were slowly motile when released from the capsule into sea water. The blastula is the earliest stage at which activity is observed in *Conus* embryos (Ostergaard, 1950).

An egg capsule opened on the second day after collection contained motile embryos which resembled early trochophores (Fig. 3*a*). The resemblance of this stage to typical trochophore larvae is somewhat closer in *C. abbreviatus* than in other species of *Conus* (see below and Ostergaard, 1950) in which the trochophore stage is suppressed. This is typical of gastropods in which this stage is passed within an egg capsule or brood pouch (Raven, 1958).

The trochophore-like embryos of *C. abbreviatus* measured 270 × 170 μ and retained the pink color of the previous stage. On the fourth day after collection, the first traces of shell were visible (Fig. 3*b*). Two days later, velar lobes were beginning to form (Fig. 3*c*) and the embryos, which now measured 255 × 150–165 μ, swam actively when released from the capsule. On the 9th day after collection the velar lobes and larval shell appeared to be fully developed (Fig. 3*d–f*). The pink color persisted, and the shells were transparent and bore numerous fine nodules (Fig. 3*d, e*). Otocysts, eyes, heart, foot, and operculum were easily visible. The maximum dimension of the shell was 270 μ, and the breadth of the velar lobes was about 250 μ. The veligers were motile within the capsule and swam actively when released from it into sea water. No further development was detected on the 11th day after collection. On the 13th day after collection, presumably the 14th or 15th day after oviposition, some veligers hatched from egg capsules following apparent dissolution of the exit window. The hatched veliger larvae closely resembled those in Figure 3 *d–f* but measured 275–300 μ in maximum shell dimension. They swam freely in the container and were colorless except for a row of green pigment spots inside the margins of the velar lobes (Fig. 3*e, f*). Unhatched veligers within the capsule retained the pink color in the visceral mass.

FIG. 2*a*. Egg capsules of *Conus abbreviatus* Reeve. Diamond Head, Oahu, 10 March 1956. Portion of a row of capsules affixed to metal can lid.

Within two days the rest of the veligers appeared to have hatched from the capsules. During the ensuing 10 days no further development was observed and none of the larvae settled to the bottom of the container to assume the benthic mode of life. By the 11th day after hatching, all of the larvae were dead and the observations were terminated. The course of larval development is summarized and compared with that of other species in Table 3.

Conus catus Hwass *in* Bruguière

A specimen of *C. catus* (no. 2180) collected at Lower Paia, Maui, on 5 August 1956, was observed to have affixed a single egg capsule to its shell about 2 hr. after collection. The capsule measured 12 × 10 mm.

On 22 August 1956, a female *C. catus* (no.

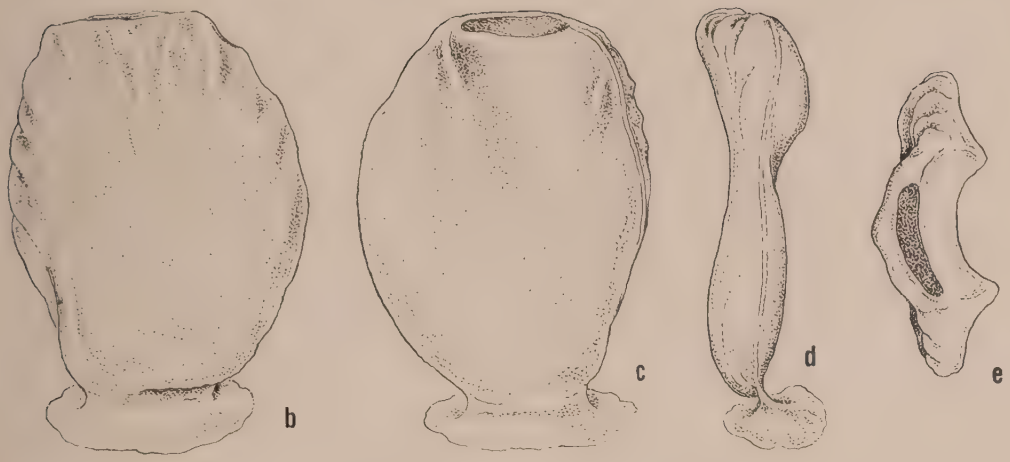


FIG. 2*b–e*. *b,c,d*, Side and edge views of one capsule; *e*, top view of another capsule.

2299, 40 × 26 mm.) was found ovipositing at Koko Head Sandy Beach, Oahu, by M. A. Kohn. Three capsules had been deposited, and spawning probably would have continued had not the adult been disturbed. However, a second cluster of 4 essentially identical capsules, unaccompanied by an adult, was also collected (Table 1). Ostergaard (1950) figured 19 capsules in an egg mass of *C. catus*.

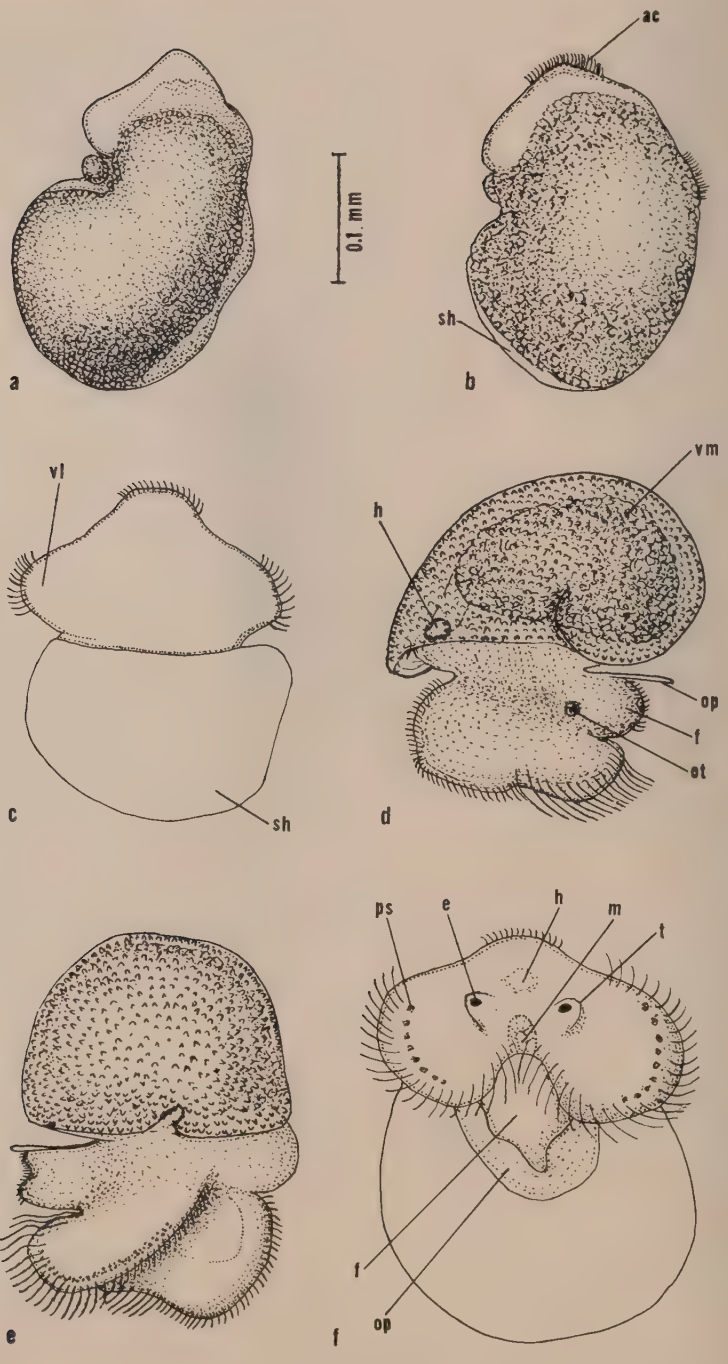
The form of the egg capsules of *Conus catus* is typical, although the stalk is somewhat taller than in other species of the genus (Fig. 4). The lateral edges are slightly convex; the upper edge is quite straight. Capsules in both clusters contained embryos in the 4-cell stage which measured 250–300 μ across. A few uncleaved eggs 235 μ in diameter were present in one capsule. The characteristics of these egg masses are sum-

TABLE 2

NUMBER OF EGGS PRODUCED, EGG DIAMETER, LENGTH OF DEVELOPMENT BEFORE HATCHING, AND STAGE AT HATCHING OF *Conus* IN HAWAII

SPECIES	AVERAGE NO. EGGS/CAPSULE	AVERAGE NO. EGGS/EGG MASS	AVERAGE EGG DIAMETER (μ)	TIME OF DEVELOPMENT IN EGG CAPSULE (days)	STAGE AND SIZE AT HATCHING	
abbreviatus	1,300	44,000	200	14	veliger	270μ
catus	1,650	15,000[1]	220	15–16[1]	veliger	375μ[1]
ebraeus	——	——	——	——	veliger[1]	280μ[1]
imperialis	5,900	(35,400)	225	——	——	——
leopardus	2,950	56,000	——	——	veliger	360μ
lividus (?)	——	——	——	——	veliger	250μ
pennaceus[2]	80	3,500	460	16[1]	veliconcha	1200μ
quercinus	9,700	210,000	215	15–16	veliger	285μ
rattus	2,000[1]	44,000[1]	125[1]	——	(?)veliger	240μ
vexillum[3]	"thousands"[1]	——	140[1]	12[1]	veliger	250μ[1]
vitulinus	——	——	225	14–15	veliger	360μ

[1] Data from Ostergaard (1950).
[2] This species is referred to as *C. omaria* by Ostergaard (1950).
[3] This species is referred to as *C. sumatrensis* by Ostergaard (1950).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marized in Tables 1 and 2. It was not possible to follow the course of larval development, but Ostergaard (1950) has described the development of *C. catus* in some detail.

Conus imperialis Linnaeus

A female *C. imperialis* (no. 1983, 79 × 23 mm.) was observed ovipositing in a depression on the side of a large dead coral head in 0.3 m. of water at Diamond Head, Oahu, on 6 July 1956. Six capsules had been deposited when the rock was turned over and oviposition was interrupted. The capsules are rather long and narrow, measuring about 19 × 13 mm., and do not rise vertically from the stalk and basal plate but are skewed to one side (Fig. 5). Upon return to the laboratory, one of the capsules was pierced and some of its contents withdrawn with a pipette. The eggs were uncleaved and measured 225 μ in diameter. Eggs in the same capsule had not undergone the first cleavage 3.7 hr. later. Twenty-four hr. later, however, eggs in the same capsule had progressed to the 4-cell stage, which measured 250 μ on each side and resembled the 4-cell stage of *C. quercinus* (Fig. 12*c*).

On the 3rd day after oviposition, a second capsule was examined and found to contain embryos in the blastula stage. No further development took place in the cultures and all of the embryos appeared to be dead and were fixed on the 16th day after oviposition. The egg masses and larval development, as far as it could be studied, agree closely in the same species studied in the Seychelles Islands (Kohn, MS). The characteristics of the egg mass from Oahu are summarized in Tables 1 and 2.

Conus leopardus (Röding)

A cluster of 19 very large egg capsules was found adrift in Kaneohe Bay, Oahu, at a depth of 5 m. by A. H. Banner on 25 April 1956. The basal plates of the capsules were confluent, holding the entire mass together. A bright green filamentous alga was associated with the egg

FIG. 4. Views of an egg capsule of *Conus catus* Hwass *in* Bruguière. Koko Head, Oahu, 22 August 1956.

mass when it was collected. However, the original substratum from which the mass presumably became detached is not known. The largest capsule measured 58 × 37 mm., exclusive of stalk and basal plate, and the average size was 54 × 35 mm. This is about twice as large as any other known *Conus* egg capsule. For this reason the egg mass is tentatively assigned to *C. leopardus*, which is by far the largest species in the family in Hawaii, reaching a length in excess of 20 cm. The capsules were collected in an area where *C. leopardus* is known to occur.

The capsules differ in shape from those of other species of *Conus* by having one lateral edge concave (Fig. 6). The capsular wall is roughened by numerous irregular ridges.

It was not possible to examine the contents of the egg capsules until 2 days after collection. At that time typical veliger larvae, measuring 345 μ in maximum shell dimension, were present. Thus although the adults and egg capsules of *C. leopardus* are very large, the veliger larvae, and presumably the eggs, are not significantly larger than those of much smaller species (Table 2). Indeed the veligers are much smaller than those of *C. pennaceus* (Table 3), the adults of which species are typically one-fourth or less the size of *C. leopardus*.

FIG. 3. Developmental stages of *Conus abbreviatus* Reeve. *a*, 3- or 4-day embryo, in modified trochophore stage. *b*, 5- or 6-day embryo, with first trace of shell present; *c*, 7- or 8-day embryo, showing developing velar lobes. *d*, fully-formed veliger, 10 or 11 days, lateral view; *e*, as *d*, but anterior-lateral view; *f*, as *d*, but anterior-ventral view, showing expanded velar lobes and ventral aspect of foot and operculum. *ac*, Apical cilia; *e*, eye; *f*, foot; *h*, heart; *m*, mouth; *op*, operculum; *ot*, otocyst; *ps*, pigment spot; *sh*, shell; *t*, tentacle; *vl*, velar lobe; *vm*, visceral mass.

TABLE 3

COURSE OF EMBRYONIC DEVELOPMENT IN HAWAIIAN *Conus*

DAYS AFTER SPAWNING[1]	*C. abbreviatus*	*C. pennaceus*	*C. quercinus*	*C. vitulinus*
0–1	blastula (200μ)[2]	early cleavage stages (460μ)	uncleaved eggs (200μ)	early cleavage stages (225μ)
2–3	early trochophore (270μ)		early blastula	blastula (225μ)
4–5	well-developed trochophore (240μ)	late trochophore	blastula (200μ)	
6–7	early veliger (255μ)		trochophore (240μ)	trochophore (360μ)
8–9			early veliger (315μ)	veliger (385μ)
10–11	well-developed veliger (270μ)	well-developed veliger (780μ)	well-developed veliger (275μ)	
12–13			well-developed veliger (285μ)	
14–15	hatching (veliger) (300μ)		hatching (veliger) (290μ)	hatching (veliger) (370μ)
16		hatching[3] (veliconcha) (1200μ)		
17–25	no further development			

[1] Probable in *C. abbreviatus*.
[2] Maximum dimensions are indicated in parentheses.
[3] Data from Ostergaard (1950).

Only 2,950 veligers were present in the one capsule which was counted, and they occupied but a small portion of the capsule. As in several other species, yellowish-green pigment spots about 10 μ in diameter were present inside the margins of the velar lobes. A large operculum, a pair of otocysts, each containing an otolith, and black eyes were present. The long (*ca.* 75 μ) velar cilia beat slowly and metachronally. The larval shell was transparent but covered with minute (*ca.* 2 μ) raised dark nodules. The visceral mass appeared white within the shell.

Several hours later on the same day, hatching began and the hatched veligers, which measured 360 μ in maximum shell dimension, swam about slowly in a 4-l. jar of aerated sea water. On the following day, the jar was densely populated with freely swimming veligers, which swam more rapidly than on the previous day. The population was then divided between two 4-l. jars. Three days later, most of the veligers had died. Four of those which remained alive were placed in a large Stender dish (*ca.* 120 mm. in diameter) containing sea water and sand from the reef. Of these, three continued to swim about, but one settled to the bottom, where it actively beat its cilia and possibly engaged in feeding. If disturbed, it swam actively for a few seconds, then returned to the bottom. However, it was not observed to crawl on its foot, nor was there any evidence of resorption of the velar lobes or other manifestation of metamorphosis. On the following day, all four larvae in the dish were dead. Two days later, the 6th day after hatching, a few swimming veligers remained in one jar, but the observations had to be terminated.

Conus lividus Hwass *in* Bruguière (?)

On 7 February 1956, at Maile, Oahu, two clusters of straw-colored egg capsules were found affixed to the underside of a coral rock, under which a large female *C. lividus* (no. 1264, 45 × 26 mm.) was also present. One cluster consisted of 36 egg capsules, all of which were empty, and the other contained 30 capsules, some of which contained dead veliger larvae. Since these capsules were probably deposited

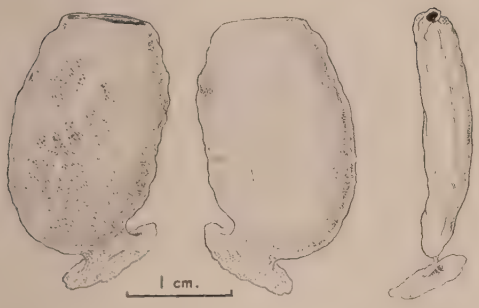

FIG. 5. Views of an egg capsule of *Conus imperialis* Linnaeus. Diamond Head, Oahu, 6 July 1956.

two or more weeks prior to their collection, it is by no means certain that the adult female associated with them was the parent. Indeed, it is not certain that the capsules are actually those of *C. lividus*, and the identification is tentative. It is supported, however, by morphological similarities with capsules known to have been deposited by *C. lividus* in other areas (Kohn, MS).

The capsules are distinguished from those of several other species of *Conus* by being somewhat more discoid in form and bearing crenations along the edges of the otherwise rather smooth capsular wall (Fig. 7). There are also two more or less parallel ridges extending downward from the corners of the exit window, as in *C. abbreviatus*. The capsules in the smaller group, shown in Figure 7, averaged 11 mm. in height and maximum breadth. The capsules in the other group were essentially similar in appearance but were slightly smaller, averaging 9.5 mm. in both dimensions.

The veliger shells, 235–260 μ in maximum dimension, were yellowish brown and bore minute densely-packed, raised, darker brown nodules over the entire surface.

Conus pennaceus Born

Egg masses of *C. pennaceus* have been collected on numerous occasions in Hawaii (Table 1). As noted by Ostergaard (1950) the egg mass of *C. pennaceus*[3] differs from those of other species in that only a few capsules are attached directly to the substratum. Most are affixed to previously deposited capsules, thus forming the "arches and bridges" mentioned by Ostergaard and making the entire mass very compact (Fig. 8). The individual capsules are typical of the genus (Fig. 9).

In the present study, 10 egg masses of *C. pennaceus* were collected on seven occasions, all in the months of June, July, and August (Table 1). The 2 egg masses reported by Ostergaard (1950)[3] were collected in May. The data suggest May–August as the breeding season of this species.

The development of *C. pennaceus* from fertilized egg to hatching has been described and illustrated by Ostergaard (1950). It was possible to follow portions of the course of larval development in 2 of the egg masses listed in Table 1.

On the day after collection, the egg mass from Diamond Head, Oahu, 10 June 1956 (which was found adrift over the reef by P. Helfrich), contained early veliger larvae which appeared to be in the later stages of torsion. A very small shell rudiment, a slowly beating heart, and eyes were present. On the 4th day after collection, the veligers appeared to be between the stages shown by Ostergaard's figures 11d and 11e, or probably about 12–13 days from oviposition. The siphonal canal of the shell had begun to form, and a row of chartreuse pigment spots was present along the margin of the velar lobes.

During the next 7 days, the shells gradually grew to 1.1 × 0.7 mm., and the wall of the exit window became thinner and more transparent, but no capsules hatched. Hatching of a few capsules finally occurred on the 16th day after collection. This probably corresponded to 25–26

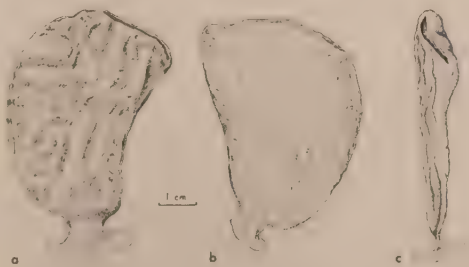

FIG. 6. Egg capsules of *Conus leopardus* (Röding). Kaneohe Bay, Oahu, 25 April 1956. *a*, Side view of one capsule; *b* and *c*, opposite side and edge views of another capsule.

[3] This species is referred to as *C. omaria* Hwass by Ostergaard (1950).

days after oviposition, an abnormally long embryonic period compared with other species and with Ostergaard's observations on *C. pennaceus*. Most of the capsules in the cluster underwent no further change and the observations were terminated.

The egg mass collected at Hauula, Oahu (Table 1) (with an adult female, no. 1811, 35 × 21 mm.) was attached to the underside of a basalt rock in less than 0.5 m. of water. On the day of collection, one capsule was removed from the cluster and found to contain larvae in the advanced veliger or veliconcha stage. The latter term was introduced (Lamy, 1933; Werner, 1939) for fully developed veligers immediately

FIG. 8. Egg mass of *Conus pennaceus* Born. Diamond Head, Oahu, 10 June 1956.

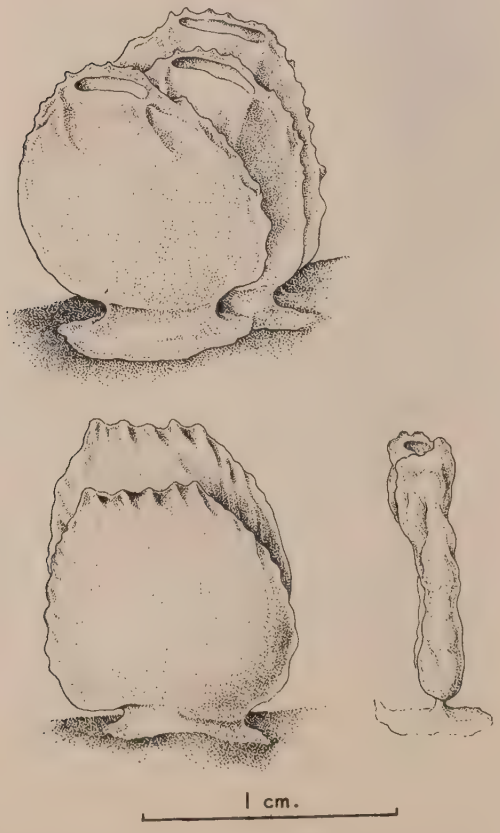

FIG. 7. Views of egg capsules tentatively assigned to *Conus lividus* Hwass *in* Bruguière. Maile, Oahu, 7 February 1956.

prior to metamorphosis. The larval shells were pale orange pink and measured 1.2–1.3 × 0.7 mm. When released from the capsule, the larvae swam with the velar lobes up, a position apparently determined by the weight of the shell. The larvae closely resembled that illustrated by Ostergaard (1950: fig. 11e). The remaining intact capsules were placed in a vessel of slowly circulating aerated sea water and a substratum of sand from the reef.

On the following day, one larva hatched from an egg capsule and, in contrast with those observed by Ostergaard, was quite able to swim about in the dish. However, its velar lobes and shell aperture always pointed upward, and the shell often rested on the substratum. The foot was not employed in locomotion. On the next day, 10 similar free-swimming veliconchas were observed in the dish. Two days later, although larvae remained in some capsules, many had hatched. A sample of these was observed to be distributed in the container as follows:

Freely swimming	0
Resting on bottom with velar lobes extended	37
Velar lobes apparently regressing	3
Settled: crawling on substratum	38
Dead: empty shells	1
Sample size	79

The later stages of larval development in *C. pennaceus* may be summarized as follows: the larvae hatch as veliconchas which may swim

freely for about 1 day. They then rest on the bottom with the velar lobes extended upward and the cilia beating. Ostergaard's observation of propulsion along the substratum by the velar cilia was confirmed. Then the velar lobes begin to be resorbed. This was observed in three larvae, in which the margins of the velum were wrinkled and the length and width of the lobes was about one-half of the earlier dimensions. These veliconchas appeared otherwise normal. On the 2nd day after hatching, the young snail begins to crawl about on its foot (Fig. 10). However, the velar lobes have not been completely resorbed by this time; they are often extended by the crawling snail. When the larva assumes the benthic mode of life, the shell becomes darker orange in color, probably due to thickening, and new shell growth is apparent along the outer lip.

Ten days after the first hatching, the shells of the largest individuals measured 1.38 × 0.80 mm. and consisted of two complete whorls. However, mortality gradually increased in the culture, no individuals advanced beyond the end of the second whorl, and on the 27th day after the first hatching only one individual remained alive and the observations were terminated. Postlarval growth of *C. pennaceus* has been discussed elsewhere (Kohn, 1959*b*).

Conus quercinus Solander

Two clusters of egg capsules of *Conus quercinus* were collected in 1–2 m. of water at Sand (Ahuolaka) Island in Kaneohe Bay, Oahu, on 9 February 1956. The larger cluster contained 40 capsules and was affixed to a large clump of the red alga, *Acanthophora orientalis* J. Agardh.

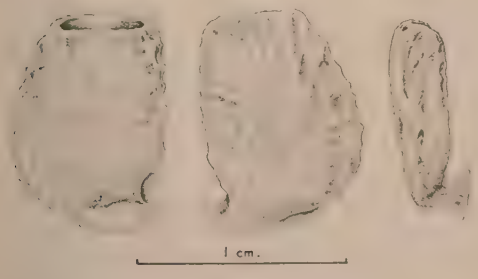

FIG. 9. Views of an egg capsule of *Conus pennaceus* Born. Diamond Head, Oahu, 10 June 1956.

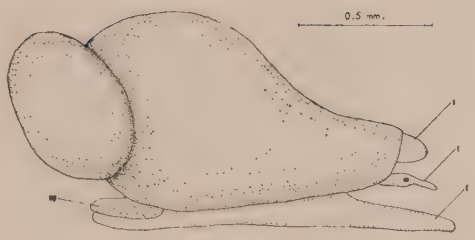

FIG. 10. Settled veliconcha of *Conus pennaceus* Born. *f*, Foot; *op*, operculum; *s*, siphon; *t*, tentacle, bearing the eye.

A pair of adult *Conus quercinus* (no. 1284, ♀, 91 × 56 mm.; no. 1285, ♂) were adjacent to the egg mass, and another pair and three single individuals were present within a radius of 1 m. *C. quercinus* appears to migrate from deeper water and to congregate for spawning in February–April of each year (Kohn, 1959*b*: 81). At this season adults are commonly found in the shallow water around Sand Island, a sand bank which is partially exposed at low tide. At other seasons they are absent from this site (Kohn, 1959*b*: fig. 29). The microhabitat of *C. quercinus* is quite distinct from those of its congeners in Hawaii (Kohn, 1959*b*). It occurs on vast sandy areas generally devoid of coral and rocks. As noted above, the egg capsules in the large mass were anchored to an alga. The other egg mass, which consisted of only three capsules, was affixed to a red sponge.

The congregation of several or many individuals associated with spawning was not observed in any other Hawaiian species of *Conus* but has been observed in other areas and will be discussed more fully elsewhere (Kohn, MS).

Most of the capsules in the large cluster were arranged in rows of up to eight and were affixed to each other by confluence of the basal plates (Fig. 11) as well as to the alga. A few were attached to previously deposited capsules, but this was not as common as in *C. pennaceus* and no bridges were present. The form of the capsules is rather irregular (Fig. 11). One wall bears numerous small ridges; the other has two more distinct ridges proceeding downward from near the corners of the exit window. The capsule is white when deposited but, since the

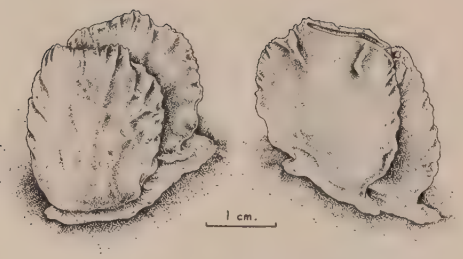

FIG. 11. Portion of a row of egg capsules of *Conus quercinus* Solander. Sand Island, Kaneohe Bay, Oahu, 9 February 1956.

cluster extends upward into the water and is not covered, it soon becomes a substratum for epiflora and epifauna. The characteristics of the egg masses of *C. quercinus* are summarized in Tables 1 and 2.

On the day of collection, one capsule was removed from the large cluster and found to contain pale pink, uncleaved, round-to-ovate eggs embedded in albuminous material and measuring 175–250 μ (Fig. 12*a*). The egg mass had probably been deposited on the same day. On the following day the first cleavage furrow was visible (Fig. 12*b*). On the 3rd day after collection, it was difficult to discern cell outlines, but the embryos, which were now white, appeared to be in the second to fourth divisions (Fig. 12*c, d*) and were motile when released from the capsule into sea water. Another capsule examined on the 5th day after collection showed little further progress. The embryos were quite motile and micromeres were observed in a few. Two days later, the embryos had increased in size to 240 $\times$ 180 μ and were in the modified trochophore stage (Fig. 12*e*).

Two days later, on the 9th day after oviposition, the embryos had grown to a maximum size of 315 $\times$ 195 μ and the shell and velar lobes had begun to form (Fig. 12*f*). Two days later, the fully developed veliger shells had attained a length of 255–295 μ. The velar lobes were well developed, and the heart, kidney (which was bright red), otocysts, eyes, and mouth (which measured 6 $\times$ 23 μ) were visible (Fig. 12*g, h, i*). The small foot rudiment bore a large operculum. The shell was brown and decorated with many rows of brown nodules, which were about 3 μ in diameter, rose 3–4 μ above the surface of the shell, and were about 3 μ apart. The growing lip of the shell was transparent.

On the 13th day after oviposition, the veligers were motile within the capsules and in many they were crowded in the vicinity of the exit window. Veligers removed from one capsule were found to have a row of green pigment spots, about 15 μ in diameter, inside the margins of the velar lobes, which had grown to about 285 μ across. The shells had not increased in size, but the outer lip was no longer transparent, indicating increased thickness. Two days later, on the 15th day after oviposition, some veligers hatched from the capsules and were swimming actively about in the container. By the following day most, and on the next day all, of the capsules had hatched.

Although the foot increased in size during the next few days, no significant over-all growth was apparent, and the velar lobes of some hatched veligers appeared reduced in size (Fig. 12*j*). None of the larvae settled to crawl on the bottom. Mortality in the culture gradually increased and only one or two larvae remained alive on the 8th day after the first hatching, when the observations were terminated.

Conus rattus Hwass *in* Bruguière (?)

A cluster of 31 egg capsules was collected from depressions in the underside of a large coral rock in 1 m. of water on the reef opposite the marine laboratory at Waikiki, Oahu, on 21 March 1956. No adult *Conus* was present in the immediate vicinity, but the capsules closely resemble those of *C. rattus* described by Ostergaard (1950).[4] They are tentatively assigned to that species, which is not uncommon on the same reef.

The capsules (Fig. 13) measured 11–15 $\times$ 10–14 mm. and most were empty. However, a few contained veliger larvae, which hatched soon after being placed in a vessel of sea water in the laboratory. The veliger shells were yellowish brown with darker brown granulations and measured 240 μ in maximum dimension. Eyes and otocysts were visible and a row of green pigment spots was present around the margins of the velar lobe. A few of the larvae remained

[4] This species is referred to as *C. tahitensis rattus* Hwass by Ostergaard (1950).

alive for 13 days after hatching, but they grew only to a length of 250 μ and no further development occurred.

Conus vitulinus Hwass *in* Bruguière

On 7 July 1956, at the reef at Ala Moana, Oahu, an adult *C. vitulinus* (no. 2020, 67 × 38 mm., sex undet.) was collected on sand under a dead coral rock, to the underside of which was attached an irregular cluster of 11 egg capsules. Some of the capsules were attached to others by confluence of the basal plates, but all were affixed to the substratum as well. They measured 23 × 16–17 mm. and their shape was typical of the genus (Fig. 14).

Eight hr. after collection, one capsule was examined and found to contain 1-, 2-, and 4-cell stages, which measured 225 μ in diameter

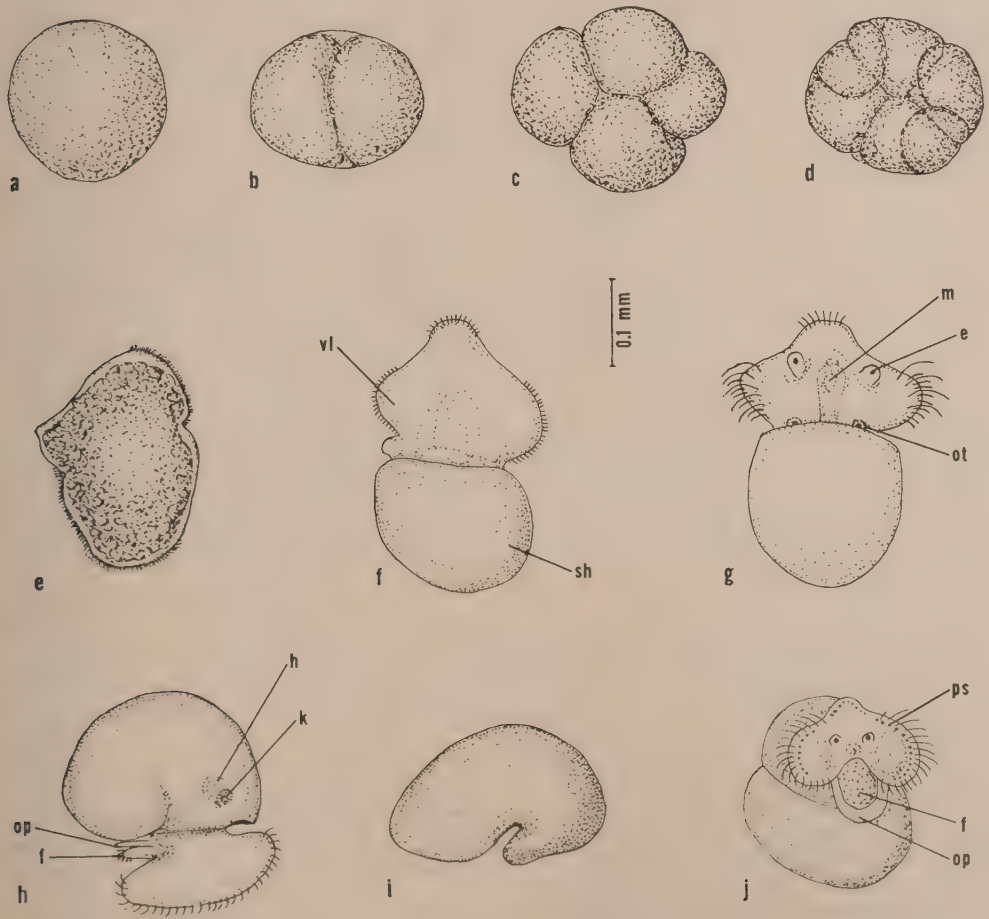

FIG. 12. Developmental stages of *Conus quercinus* Solander, from the same egg mass as the capsules illustrated in Figure 11. *a*, Uncleaved egg; *b*, 2-cell stage; *c*, 4-cell stage; *d*, embryo in third cleavage division; *e*, trochophore-like stage; *f*, early veliger; *g*, developing veliger, anterior-ventral view; *h*, developing veliger, right lateral view; *i*, shell of veliger, left lateral view; *j*, hatched, fully developed veliger. *e*, Eye; *f*, foot; *h*, heart; *k*, kidney; *m*, mouth; *op*, operculum; *ot*, otocyst; *ps*, pigment spots; *sh*, shell; *vl*, velar lobe. Nodules present on shells are not indicated in figures.

FIG. 13. Views of an egg capsule tentatively assigned to *Conus rattus* Hwass *in* Bruguière. Waikiki, Oahu, 21 March 1956.

(Fig. 15*a*, *b*). Oviposition probably occurred earlier the same day. Two days later, the embryos had progressed to the early blastula stage and macromeres and micromeres were distinctly visible (Fig. 15*c*). A capsule examined on the 6th day after collection was found to contain motile trochophore-like larvae which measured 360 × 210 μ (Fig. 15*d*). On the 9th day, the veliger stage was fully developed, the maximum dimension being 375–400 μ (Fig. 15*e*, *f*). On the 11th day, the veligers were motile within the capsules and on the following day crowding toward the exit window was observed. All of the capsules hatched during the next 3 days, or 14–16 days after oviposition. The otocysts and reddish-brown kidney were visible and green pigment spots almost filled the velar lobes of some individuals.

Some of the veligers swam about freely in the vessel, but most remained on the bottom, which had been covered with sand from the reef, and propelled themselves by beating the velar cilia. When disturbed, they swam briefly away from the source of irritation. However, none of the larvae settled to crawl on their feet, and all had died without undergoing any further growth or development by the 4th day after hatching began.

On 24 July 1957, a cluster of 12 egg capsules was found attached to the underside of a coral rock in a large tide pool at Diamond Head, Oahu. A large adult *C. vitulinus* (no. 7034, 65 × 37 mm., sex undet.) was present on the sand substratum under the rock. Upon return to the laboratory, one of the capsules was examined and found to contain eggs in 1-, 2-, and 4-cell stages; the last measured about 250 μ across. On the second day after collection, a capsule contained embryos which still measured 250 μ in diameter but had progressed to the blastula stage. The observations had to be terminated at this point, but development thus far was essentially similar to that in the egg mass described above.

DISCUSSION

Spawning site and season. Egg capsules of *Conus* have been collected on 31 known occasions in Hawaii, chiefly by Ostergaard (1950) and by the writer and colleagues. All collection records are summarized in Table 1. At least 12 species are represented.

As noted previously (Kohn, 1959*b*):

> Coral reef platforms, but not marine benches, provide suitable attachment sites for egg capsules of *Conus*. Of 36 egg masses collected in the field, 29 were recorded from reef platforms. An almost complete absence of records from marine benches suggests that spawning is unsuccessful there. This is probably due to the absence of protected pools in which egg capsules may be deposited without being subject to desiccation at low tide and/or torn away by heavy surf at high tide. Recruitment of bench populations [of adults] is probably from pelagic veliger larvae which have been carried from other areas and are washed onto marine benches in condition to settle and assume the benthic mode of life.

All of the capsules were collected between February and August, although search for them was not restricted to, or emphasized during, that period. The data suggest that most species of

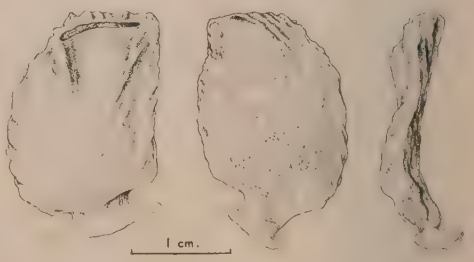

FIG. 14. Views of an egg capsule of *Conus vitulinus* Hwass in Bruguière. Ala Moana, Oahu, 7 July 1956.

Conus spawn during about the same part of the year. The spawning season of most species for which more than one egg mass has been collected is rather extended over the months cited. Capsules containing viable eggs and embryos of *C. rattus* have been collected from February to August, of *C. abbreviatus* from March to July, and of *C. pennaceus* from May to August.

The spawn of most species has been observed only rarely. However, 12 egg masses of *Conus pennaceus* were collected on nine occasions, all in the months of May, June, July, and August, suggesting that these months constitute the breeding season in this species. The preponderance of data for *C. pennaceus* (Table 1) is attributed partly to the fact that the egg masses of this species are readily identifiable, even in the absence of the parent from the collection site. Unlike other species, which attach each capsule to the substratum, *C. pennaceus* attaches only a few to the substratum, while the rest are attached to previously deposited capsules.

Development. The eggs of *Conus* are typically white or pink, spherical or nearly so, and without a surrounding membrane. Polar bodies were not observed, but Ostergaard (1950) reported their occurrence in *C. vexillum*.[5] There are no nurse eggs; all or almost all eggs may develop to hatching in nature, but development was often arrested in a usually small but variable proportion of embryos in egg masses cultured in the laboratory.

The number of eggs produced, egg diameter, length of development before hatching, and stage at hatching are summarized for 11 species of *Conus* from Hawaii in Table 2. Egg diameter was measured in 8 species, of which 7 ranged from 125 μ to 225 μ. In all of these species, the number of eggs was large, about 1,000–10,000 per egg capsule and 15,000–210,000 per egg mass (Table 2). One species, *C. pennaceus*, has much larger eggs (460 μ), of which considerably fewer are deposited (80/egg capsule; 3,500/egg mass).

As can be seen in Table 2, all the species with large numbers of small eggs hatch as free-swimming veligers, while *C. pennaceus* has virtually no pelagic stage, as was discussed in detail above. These data are in agreement with the correlations of large numbers of small eggs with long pelagic life and small numbers of large eggs with nonpelagic development first demonstrated by Thorson (1950, 1952) for mollusks and other benthic marine invertebrates.

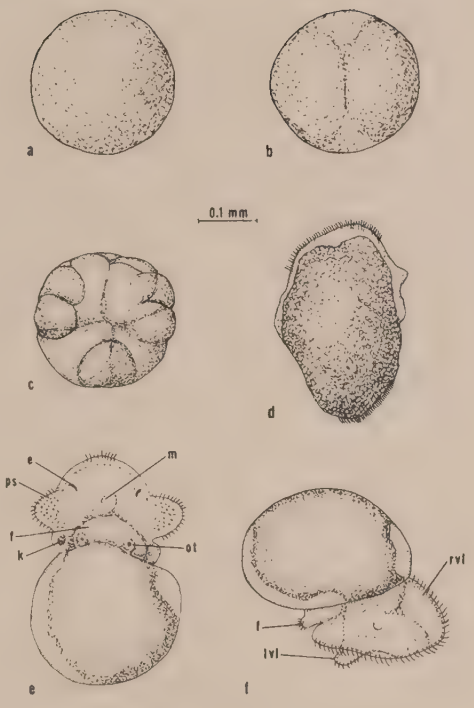

FIG. 15. Developmental stages of *Conus vitulinus* Hwass *in* Bruguière, from the same egg mass as the capsule illustrated in Figure 14. *a*, Uncleaved egg; *b*, 2-cell stage; *c*, early blastula; *d*, trochophore-like stage; *e*, veliger, anterior-ventral view; *f*, veliger, lateral view. *e*, Eye; *f*, foot; *k*, kidney; *lvl*, left velar lobe; *m*, mouth; *ot*, otocyst; *ps*, pigment spot; *rvl*, right velar lobe.

Salient features of development in four species of *Conus* are summarized in Table 3. The eggs are fertilized but have not yet undergone first cleavage when enclosed in the egg capsules. In the species studied, the trochophore stage is entered at 2–6 days, and the veliger stage at 6–10 days, after spawning. Larvae hatch as veligers about 2 weeks after spawning. These observations are in agreement with those of Ostergaard (1950), who also reported on development in

[5] This species is referred to as *C. sumatrensis* Hwass by Ostergaard (1950).

four other species, which hatched 12–16 days after spawning.

In laboratory cultures, many of the veligers (up to 75 per cent) did not succeed in escaping from the capsules. However, this may have been due to the abnormal spatial position of the detached capsules; it is less likely to occur in nature. It has been noted above that the capsules are usually affixed to the underside of a rock. Since the exit window is thus oriented downward in nature, escape of the larvae from the capsule may be facilitated by gravity after disappearance of the membrane covering the exit window. However, in laboratory cultures in which capsules were oriented as shown in Figure 1, larvae ready to hatch were often observed crowded toward the exit window before dissolution of the membrane, and many escaped from the capsule by actively swimming upward. These observations suggest that positive or negative geotropism is not a factor in the hatching of *Conus* larvae.

The length of the pelagic stage could not be determined except for *C. pennaceus*, in which it is only 1 day or less. The maximum survival time of free-swimming veligers in the writer's laboratory was 13 days (*C. rattus*). Partial metamorphosis was observed only in *C. pennaceus*.

The nature of the food of the larval stages is unknown. Protozoa abounded in the cultures. Thorson (1946) concluded that all prosobranch larvae known from the Øresund feed on phytoplankton, and he calculated the theoretical maximum diameter of the food to be 5–45 μ. The mouths of *Conus* veligers measured were of about the same diameter as the esophagus of the smaller larvae measured by Thorson. Thus the larvae of *Conus* probably depend for food on phytoplankton, nannoplankton, and detritus. Examination of squash preparations of *C. pennaceus* a few days after settling revealed the presence of radula teeth. These differ in form from the adult teeth, being shorter in proportion to the thickness, and they are probably not functional. The method of feeding, as well as the food, is thus not known at this stage of the life history. The rather fragmentary information available on post-larval development and growth of *Conus* in Hawaii has been reported elsewhere (Kohn, 1959*b*).

SUMMARY

Egg capsules of 9 of the 33 species of *Conus* found in Hawaii are described and figured. Interspecific variation is usually not sufficient to permit identification of the species to which an egg mass belongs in the absence of the parent.

The complete course of larval development within the egg capsule from spawning (uncleaved egg) to hatching (veliger or veliconcha) is described for 4 species. Early cleavage stages occur 1–3 days after spawning, suppressed trochophore stage at 2–6 days, and veliger stage at 6–10 days. Freely swimming veligers hatched from egg capsules of 3 species 14–15 days after oviposition, in agreement with the developmental time scale of other species of *Conus* previously reported on by Ostergaard (1950).

All species found to have pelagic larval stages produced large numbers (15,000–210,000) of small (125–225 μ) eggs. It was not possible to determine the length of the free-swimming stage in any of these species.

Hatching in one species, *C. pennaceus*, occurs 16–26 days after oviposition at the advanced veliger or veliconcha stage. The larvae swim about for 1 day or less and then assume the benthic habit and begin metamorphosis. This species produces small numbers (3,500) of large (460 μ) eggs. This characteristic is correlated with the absence of a long pelagic larval stage.

ACKNOWLEDGMENTS

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A Contribution to the Biology of the Convict Surgeonfish of the Hawaiian Islands, *Acanthurus triostegus sandvicensis*

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A Contribution to the Biology of the Convict Surgeonfish of the Hawaiian Islands, *Acanthurus triostegus sandvicensis*[1]

JOHN E. RANDALL[2]

THE SURGEONFISHES (family Acanthuridae), which are distinctive chiefly in their possession of a knifelike spine or spines at the base of the tail, are one of the dominant groups of tropical inshore marine fishes. Over much of their vast range, including Hawaii, where they are especially prominent on the reefs, they are important components of subsistence fisheries. Little is known of their biology, however. There are only scattered references to the herbivorous food habits of the group and general remarks on the habitat of certain species.

An analysis of the generic classification of the family and taxonomic revisions of some of the genera have been published (Randall, 1955 *a*, *c*, *d*; 1956*b*). The largest genus, *Acanthurus*, contains 32 species, 4 of which occur in the Atlantic, and the rest in the Indo-Pacific. The present paper constitutes a report of a study made during 1952–55 upon the life history and the ecology of one Hawaiian subspecies of this genus, *A. triostegus sandvicensis*, the convict tang or convict surgeonfish. In Hawaii and elsewhere in Polynesia this surgeonfish is known as the manini, and hereafter it will usually be referred to by that name.

The manini is the most abundant species of surgeonfish in the Hawaiian Islands and commercially the most important. Judging from its prevalence in museum collections, it is also common elsewhere in the Indo-Pacific region. Jordan and Seale (1906: 354) wrote, "This species is the most abundant of the genus about Samoa, swarming everywhere on the reefs." The young reside in tidepools, and are therefore more accessible for observation and experimentation than the young of other acanthurids in Hawaii which are usually found in deeper water. The species has the widest distribution of all of the surgeonfishes—East Africa to the Gulf of California (a single record from West Africa by Fowler, 1936, should be confirmed). Thus interest in its biology may be greater than that of a localized species.

A. triostegus (Fig. 1) has been described under 10 different scientific names and placed in six different genera. Nomenclatural considerations and description of the species and variants, with special reference to fin-ray counts and coloration, have been dealt with previously (Randall, 1956*b*).

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FIG. 1. Adult manini, *Acanthurus triostegus sandvicensis*, from Hawaii. Standard length, 154 mm.

HABITAT

In the Hawaiian Islands the manini occupies a diversity of habitats. It occurs in bays, harbors, and exposed reef areas. It abounds in tide pools and shallow water, yet is known at depths of at least 100 ft. Areas of very turbid and brackish water are usually avoided, but a few manini have been collected even in these regions.

Like most reef fishes, the manini avoids extensive stretches of sand or mud bottom, preferring instead the nearness of holes or crevices in the reef for shelter and solid bottom for the growth of its algal food.

The manini appears to be more restricted in habitat in the Gilbert and Marshall islands, possibly because of the sharper definition of the ecological zones of these atolls. It occurs in these atolls both in the lagoon and outer reef areas; however it seems to be confined to relatively shallow water. On the outer reef the fish were seen only on the reef flat, the young in great abundance in the shallower sections and the adults on the seaward portions (for data from a reef-flat transect in the Gilbert Islands, see Randall, 1955*b*: 181). The species was only rarely observed in the turbulent surge channel zone and was never seen on the coralliferous terrace (benched area of living coral offshore from the reef flat).

As in the Gilberts and Marshalls, the presence of heavy coralline areas in the Hawaiian Islands seems to limit the manini. This is apparent from the underwater transect work of Brock (1954). Areas like the northern part of Kealakekua Bay, Hawaii, where the coral growth is extremely rich for the Hawaiian region, are almost devoid of manini. Since this species is herbivorous, its diminution in coral areas may be associated with the small amount of substratum available for the growth of algae. Surprisingly, other surgeonfishes such as *Acanthurus leucopareius*, *A. achilles*, *Zebrasoma flavescens*, and *Ctenochaetus strigosus*, usually less abundant than manini, are the most common fishes in this part of the bay. With the exception of *C. strigosus* which is a detritus feeder (Randall, 1955*d*), these species are more inclined to crop close to the substratum than the manini, judging from the greater amount of inorganic debris in their stomach contents, and they may therefore be superior competitors under such conditions.

The eggs and larvae of the manini are pelagic. This has been ascertained by a study of development following artificial fertilization of the eggs, by the capture of larvae in plankton nets, and by the taking of the late postlarval or acronurus stage at night lights offshore.

A total of 11 acanthurid larvae (at least 3 of which are manini), 4.2–8.7 mm. in total length, were found in the plankton collections from Hawaiian waters of Cruises 4 (May 1950) and 6 (August 1950) of the "Hugh M. Smith," a POFI research vessel. These fish were taken at eight stations ranging from about 10 to 140 mi. from the nearest island (for more exact locations, see King and Hida, 1954: fig. 1, stations 1A, 10, 13, 15, 17, and 23 of Cruise 4 and stations 7 and 14 of Cruise 6). They were captured in horizontal tows made with fine-meshed plankton nets (1 m.) which sampled three different levels simultaneously. For both cruises combined, a total of 58 hauls were made at the surface, 24 at a depth of 50 m., and 112 at depths from 100 to 300 m. Six of the larvae were taken at the surface, 5 at 50 m., and none at greater depths. The failure to find more larvae in these collections is probably due to the distance from land of most of the stations. Only 6 of the 29 stations of each cruise were within 25 mi. of any of the Hawaiian Islands and only 1 within 10 mi. The absence of larval *Acanthurus* longer than 8.7 mm. in the collections is probably due to the ability of larger larvae to elude

the plankton net. The lack of specimens shorter than 4.2 mm. may be the result of a paucity of small larvae so far offshore.

That acanthurid larvae, in general, are more abundant close to land has been shown by Reintjes and King (1953). These authors found a total of 1,067 acanthurid larvae in the stomach of 184 of 1,097 yellowfin tuna (*Thunnus macropterus*) sampled from the region of the Line and Phoenix islands. They stated that the acanthurids were common in the stomachs of nearshore yellowfin, along with balistids and carangids. Representatives of the Bramidae, Exocoetidae, and Gempylidae predominated in the stomachs of offshore yellowfin.

King kindly allowed me to examine the stomach content work sheets in order to compare the occurrence of larval acanthurids in the stomachs of tuna caught at the surface by pole-and-line fishing and by trolling with those caught at depths of about 30 to 160 mi. by long-lining. The comparison revealed more acanthurid larvae in the stomachs of surface-caught fish.

The acronurus larval form of the manini, averaging about 26 mm. in standard length in the Hawaiian Islands, leaves the pelagic realm and enters very shallow water, often tide pools, to transform to the juvenile stage. Although juveniles tend to remain in shallow water, there is a progressive movement with increasing size to somewhat deeper water. Adults are not entirely absent from tide pools, however. Several were seen in pools 1–3 ft. deep cut off from the open sea at low tide on Moku Manu and Manana (Rabbit) islands. Also, at night on Oahu, manini as large as 70 mm. were occasionally found in high tide pools.

TOLERANCE TO TEMPERATURE AND SALINITY

In order to determine if the manini is capable of surviving the extremes of temperatures and salinity which it encounters on Oahu, a survey of these factors in tide pools and brackish areas known to be penetrated by the species was undertaken and compared with the tolerance of the species to these factors as determined by laboratory experiments on both juveniles and adults.

Environmental Extremes

The survey of environmental temperature and salinity was not made on any regular schedule. Only when conditions occurred which suggested that high and low values of temperature and salinity might be found, were measurements of these factors made. Temperatures were taken at mid-depth of the tide pools tested. Water samples from which chlorinity was determined were obtained after stirring the pools. The Mohr method was used to determine chlorinity, and the results are expressed as grams of chlorine per kilogram of sea water (‰).

The maximum temperature, 35.1° C., was recorded in a tide pool with basalt bottom (almost black) at Makapuu Point on August 31, 1953, at 2:00 P.M. A reverse stratification of temperature existed in the pool at that time. A +0.2 low tide had occurred at 1:50 P.M. The maximum air temperature at Makapuu Point for August 31 was 79° F. (26.2° C.). Other fishes observed in the tide pool along with manini included aholehole (*Kuhlia sandvicensis*) and kupipi (*Abudefduf sordidus*).

The minimum temperature, 16.2° C., was recorded in a small tide pool at Diamond Head on March 5, 1954, at 11:15 P.M. A –0.2 low tide had occurred at Honolulu at 10:42 P.M. The minimum air temperature during the night was 60° F. (15.5° C.). A strong northerly wind was blowing. The wind velocity for the hour prior to the temperature reading was as high as 33 knots.

The extremes of environmental temperature recorded by Tester and Takata (1953: 48) for the aholehole on Oahu are 20.1° C. and 32.1° C. Like the manini, young aholehole are tide-pool residents. Although found in the highest pools of the intertidal zone, they are less inclined than the manini to enter small pools. A more notable difference of these two species is the ability of the aholehole to live in streams with little or no salt content.

The lowest chlorinity recorded from pools where manini were observed was 2.65 ‰. This reading was obtained from a high tide pool at Diamond Head during a heavy rain at 9:00 A.M. on March 1, 1954. A +0.1 low tide occurred at 9:02 A.M. A rivulet of rain water was observed entering the pool.

Another low chlorinity (3.62 ‰) was obtained in a sample taken from the shore of the Ala Wai Canal at the Ala Moana Bridge on February 23, 1954, following a heavy rain. Although manini could not be seen at the time because of the turbidity of the water, they were observed in the area prior to the rain and after the water had cleared.

The highest chlorinity was 21.30 ‰. The water sample was taken from the pool at Makapuu Point in which the 35.1° C. temperature was recorded.

Experiments on Temperature Tolerance

The experiments on temperature tolerance were performed on manini which were maintained in aquaria at a near-constant temperature of 24° C. for at least 24 hr. The fish were placed singly in a gallon jar of sea water at the temperature of the aquarium from which they were taken. The jar was then lowered into a water-filled copper compartment containing either a heating unit or a refrigerating unit and brought to the test temperature in 1 hr. ± 10 min. The fish were kept at this temperature for 1 hr. If death ensued before the end of this hour, the time was recorded. The duration of the test period was chosen as a rough approximation of the temporal conditions of exposure to temperature extremes experienced by manini in high tide pools. Throughout the tests the jar was strongly aerated.

At about 5° C. before the maximum or minimum temperatures were reached, the fish began to increase their rate of swimming and darted around the jar. As the lethal temperature was approached more closely, the equilibrium of the fish was affected. They swam on one side, upside down, or in small circles. The last major activity was usually a rapid, spasmodic, swimming movement, often in a short spiral.

The results, except those of tests of both juveniles and adults run at temperatures of 36.5° C. or less and 13° C. or greater (which caused no deaths), are shown in Table 1.

Although the data are not adequate to determine individual variation in the region of the temperature extremes, it seems evident that a range of about 13° to 36° C. is withstood by this species in the Hawaiian Islands, disregarding the possible extension of this range by acclimatization.

TABLE 1

TEMPERATURE TOLERANCE OF *Acanthurus triostegus sandvicensis*

STANDARD LENGTH (mm.)	TEST TEMP. (° C.)	MINUTES TEST TEMP. ENDURED
96	37.0	45
97	37.0	60
137	37.5	25
96	37.6	10
86	38.0	4
97	38.0	15
101	38.2	6
103	38.3	20
26	36.8	40
27	37.2	60
28	37.5	60
34	37.6	15
29	37.8	60
28	38.0	60
29	38.0	18
28	38.1	2
103	13.3	60
95	12.2	60
140	10.1	10
122	9.0	2
25	13.6	2
27	12.8	10
27	12.5	60
28	11.6	5
27	11.0	60
32	10.8	8

Although the range of temperature which manini can withstand appears to be extralimital to the extremes normally encountered by the species in the Hawaiian Islands, information supplied by D. W. Strasburg in a letter suggests that juvenile manini in the southern Marshall Islands are, upon occasions, killed in tide pools by heat. On August 17, 1950, a high tide pool, about 30 sq. ft. in surface area and 1 ft. deep, on the ocean side of Arno Atoll was observed at low tide to contain two small *Acanthurus triostegus triostegus* and one small *Istiblennius edentulus*. Several hours later the two manini were dead. The blenny was still living. The temperature of the pool at mid-depth was 41° C.

Experiments on Salinity Tolerance

Salinity tolerance experiments on manini were carried out in a 30 gal. aquarium. All fish were retained in aquaria at least 24 hr. before use in experiments. The fish were not exposed to the test salinity suddenly. Instead the salinity was gradually changed over a period of 2 hr. either by the removal of aquarium water and replacement with tap water (previously allowed to stand for at least 12 hr.) or the addition of salt from evaporated sea water. Fish were held at the test salinity for a maximum of 24 hr. The long period of exposure to salinity extremes was chosen because manini entering brackish areas may be subjected to water of low salt content for at least this length of time.

The results are given in Table 2. Omitted are trials run at chlorinity values greater than 1.4‰, none of which caused any deaths. No trials were run at chlorinities greater than 38.25 ‰.

Although more data are needed to determine with accuracy the minimum salinity which manini can withstand, it seems evident that manini in tide pools can tolerate a greater range in salinity than they normally experience. Since they cannot live in water of extremely low salinity, they probably do not enter fresh-water habitats.

PREDATORS

Predation on the manini is probably most acute during the early stages of the life history, but I have no information on the identity of the many pelagic animals that must feed on the eggs and small larvae. The large number of post-larval stages of acanthurids found in the stomachs of adult yellowfin tuna has been discussed.

Only a few instances of predation on juvenile manini have been encountered incidentally and are presented here briefly. Juvenile manini have been found in the stomachs of moray eels (Muraenidae). A small lizard fish (Synodidae) was observed to catch a juvenile manini in its jaws in shallow water in Kaneohe Bay, Oahu. The manini was too large to be swallowed, and it eventually escaped. A 50 mm. specimen of *Antennarius* was placed in an aquarium with six juvenile manini. A half hour later it had eaten one of them (length, 31 mm.).

In the Society Islands a juvenile *Caranx melampygus* about 90 mm. long was observed from shore to capture a small *Acanthurus triostegus triostegus* 25–30 mm. in length. This carangid occurs in the Hawaiian Islands where it probably feeds in part on manini, as may other species of *Caranx*.

Also in the Society Islands, the young *Acanthurus triostegus triostegus* have been found in the stomachs of the groupers *Epinephelus merra* and *Cephalopholis argus* and the snapper *Lutjanus vaigiensis* (Randall and Brock, 1960).

It is believed that predation (man excluded) on the juvenile stage of the manini in the Hawaiian Islands is much more pronounced than on the adult stage. In addition to the fishes mentioned above, holocentrids, scorpaenids, cirrhitids, and sphyraenids, and possibly also certain

TABLE 2

SALINITY TOLERANCE OF *Acanthurus triostegus sandvicensis*

NO. OF FISH	STANDARD LENGTH (mm.)	CHLORINITY (0/00)	NO. SURVIVING 24 HR.	MAXIMUM HR. SURVIVED
3	26–29	1.4	3	
4	26–29	0.7	0	5
3	27–29	0.1	0	less than 5
1	92	1.4	1	
1	89	0.7	0	8
2	86–90	0.4	0	between 3 and 15
3	27–29	34.8	2	
4	26–28	35.7	2	
3	28–29	37.4	2	
4	26–29	38.25	0	5
1	83	38.25	0	17.5

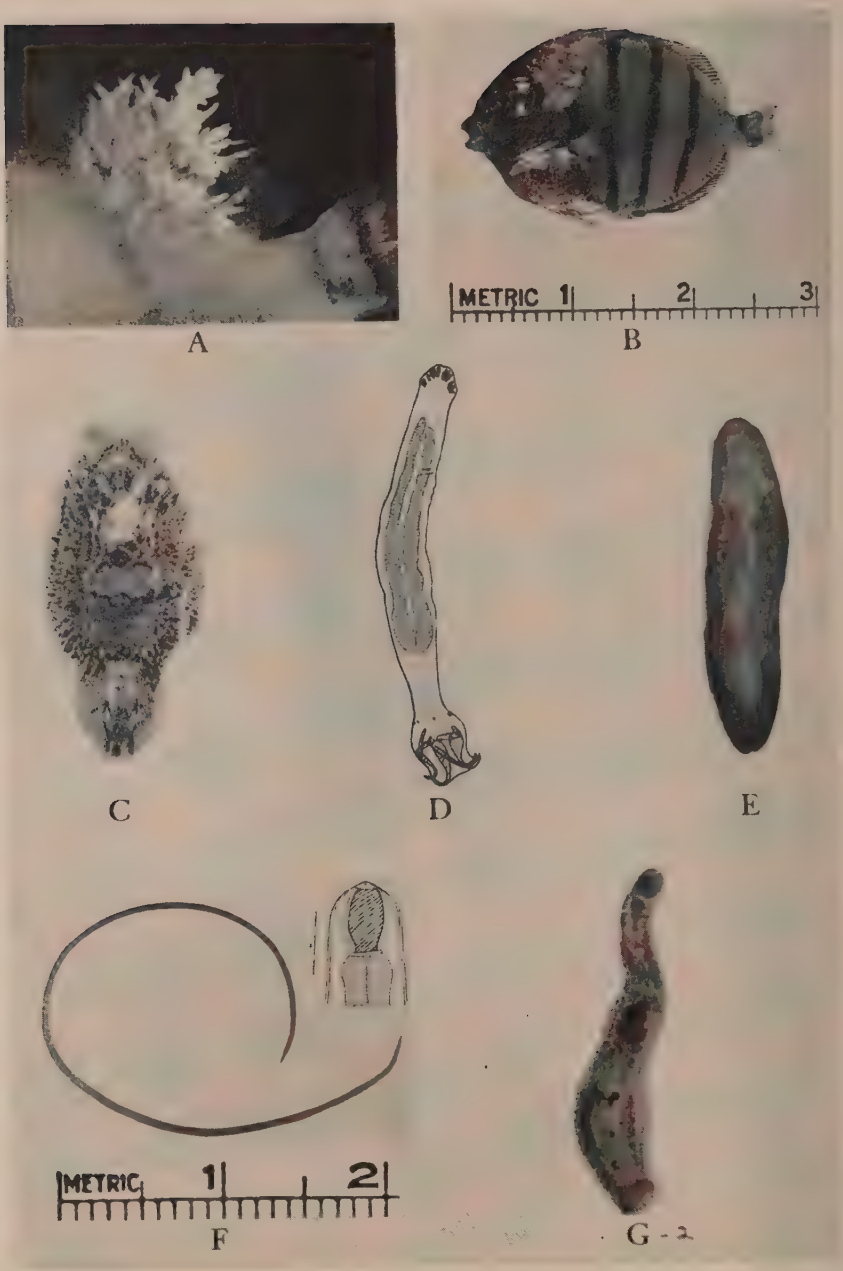

FIG. 2. Parasites of *Acanthurus triostegus sandvicensis*. *A*, Colony of *Hydrichthys* sp. on the dorsal fin of a manini in its first day of transformation from the acronurus to the juvenile state. *B*, *Hydrichthys* on the post-orbital part of the head of a manini in its second day of transformation. *C*. *Benedenia* sp. from the epidermis of an adult manini; length of trematode, 1.9 mm. *D*. *Ancyrocephalus* sp. from the gills of manini; length of trematode, 0.63 mm. *E*. *Hapladena varia* Linton from the duodenum of an adult manini; length of trematode, 2.9 mm. *F*. Adult female *Spirocamallanus monotaxis* Olsen from the intestine of adult manini; insert shows anterior end of the nematode (after Olsen, 1952); red in life, the females attain a length of about 45 mm. and the males about half this size. *G*. Unidentified leech (possibly *Johanssonia* sp.) from the gills of a manini; length of leech, 2.4 mm.

of the larger nocturnal crabs, may feed on small manini. Once a manini reaches adult size, it probably enjoys freedom from predation by most of these fishes in Hawaii, for the inshore marine fauna is peculiar in the paucity of moderately large carnivorous fishes. Shallow-water serranids and lutjanids, many of which are well adapted to prey on reef fishes, were almost totally lacking in the Hawaiian Islands until the recent effort to introduce some of these fishes. The larger native predaceous fishes, such as adults of *Caranx*, *Sphyraena*, and sharks, are not numerous at the present time, probably because of fishing pressure.

PARASITES

Seventeen species of parasites were detected on and within the manini.

Seven specimens of transforming manini were found to be parasitized by *Hydrichthys* (Fig. 2*A*), probably *H. mirus* Fewkes. This unusual hydroid occurred on the head, body, and fins and apparently does not have a preferred attachment site. It was never seen on juveniles or adults. Probably it flourishes only on postlarval manini, for it appears to be abraded and regressing on most of the transforming fish (Fig. 2*B*).

Three specimens of a monogenetic fluke (*Benedenia* sp.) were discovered on the epidermis of two adult manini. The flukes are small (less than 2 mm. in length), perfectly transparent in life, and were hidden in the slime of the fish. They are difficult to find and are probably more common than the few specimens would indicate. Figure 2*C* is a photograph of a stained specimen. Jahn and Kuhn (1932) have worked out the life cycle of *Benedenia melleni*.

The most common fluke of the manini is an unidentified species of the gyrodactylid genus *Ancyrocephalus*. Figure 2*D* is a drawing of a living specimen, 631 micra in length, which was obtained from the gills of a 132 mm. manini. This very small species of trematode was found on the gills of most of the manini which were examined. In some of the manini the infestations were heavy, an average of about one fluke per gill filament being present. It is extremely active and highly extensible and was observed to change its position on a gill filament by attaching with the anterior end and looping the posterior end over to another location where the hooks were dug into place. Siwak (1931) reported on the life cycle of a species of *Ancyrocephalus*.

Two digenetic trematodes were found in the digestive tract of the manini, *Hapladena varia* Linton and *Haplosplanchnus obtusus* (Linton), previously known only from the surgeonfishes *Acanthurus hepatus* (= *chirurgus*) and *Acanthurus coeruleus* at Tortugas, Florida. Manter (1955: 76) included a discussion of this distribution in a recent paper reviewing the zoogeography of trematodes of marine fishes.

Hapladena varia (Fig. 2*E*) was only occasionally found in the intestine of the manini. A total of 22 specimens, usually less than 3 mm. in length, were taken from 200 manini whose digestive tracts were examined for parasites. The manini harboring the flukes ranged from 43 to 136 mm. in standard length.

Haplosplanchnus obtusus was found only in juvenile manini, although it may have been overlooked in adults. It is smaller than *Hapladena varia*, usually less than 1.3 mm., and not as slender. Some of the juvenile manini contained a large number of these flukes. Seventy-one were counted in one 49 mm. specimen. Most of the flukes were in the duodenum, a few extended as far back as the halfway point of the intestine, and a few were found in the pyloric caeca.

Four different nematodes were found in the manini. Two could not be identified to genus. One of these was a very attenuate immature male from suprapharyngeal tissue of the fish. The other was represented only by larval specimens from the pyloric caeca. A third worm, which was occasionally found encysted in the mesenteries, was considered to be the immature female of an unidentified species of *Contracaecum*.

The fourth nematode, *Spirocamallanus monotaxis* (Fig. 2*F*), was described by Olsen (1952) from the sparid fish *Monotaxis grandoculis*, reportedly a mollusk-feeder. In all probability this roundworm will be found in other Hawaiian fishes.

Of 200 manini (mostly adults) from Oahu examined throughout the year for internal parasites, 76 were found which harbored *S. monotaxis* in the intestine or pyloric caeca. The av-

erage number of worms found in these 76 fishes was 5. The largest number of worms in a single fish was 35. No obvious variation of the degree of infestation of this nematode in the manini was observed during the year.

Two different leeches were found on the manini. Both are species in the family of Pisicolidae (possibly genus *Johanssonia*). One is represented by a single 2.4 mm. immature specimen (stained and photographed herein as Fig. 2*G*) which was taken from the gills of an adult manini. Six specimens of the other leech were collected from the body surface of three juvenile manini, 26–31 mm. in standard length, taken in the Ala Wai Yacht Basin on April 17, 1955. These leeches varied from 2.9 to 7.5 mm. in length, were dark brown in life, and easily detected on the host.

Six species of parasitic copepods have been taken from the body surface and pharyngeal cavity of the manini. *Lepeophtheirus dissimulatus* Wilson was the species most commonly encountered: 112 of 164 adult manini examined for parasites throughout the year were infested with from 1 to 20 of these copepods. The other parasitic copepods found were two species of *Caligus*, a *Dentigryps* sp., *Peniculus minuticaudae* Shiino, and *Nessipus costatus* Wilson, which encysts in the fins. A comprehensive report on these and other parasitic copepods from Hawaiian acanthurid fishes is being prepared by A. Lewis.

A mutualistic association was observed between the manini (and other reef fishes) and a small colorful wrasse, *Labroides phthirophagus* Randall. In the clear water off Manana Island two adult manini were seen being pecked over the head and body by this wrasse. The manini remained motionless in the water except for slight undulation of the fins. Since the stomach contents of several of the wrasses consisted primarily of calagoid copepods, it is believed that the fish were removing crustacean ectoparasites from the manini. On another occasion a manini was observed to swim directly to the "domain" of two of these labrids. As the manini was approached by one of them the surgeonfish was speared and brought ashore. Two adults of *Lepeophtheirus dissimulatus* were visible on its body.

DISEASES

The only data on disease were obtained from a series of unsuccessful attempts to maintain manini in an aquarium. Four adult fish were placed in a 23 gal. stainless steel aquarium which had previously been used to keep adult and juvenile manini for periods of several weeks. The water was aerated and filtered. Two days later two of the fish died, and on the following day the remaining fish were dead. Several hours before their death the manini swam in a disoriented manner. The fins were badly eroded. The head and body were excessively slimy, and large scattered sores were present. The fish were examined for parasites and found to have no more than the average complement.

The tank was cleaned (but not sterilized) and two more adult fish added. They died in a similar fashion in 48 hr. The tank was again cleaned and a 57 mm. juvenile manini placed in it. This fish died just 48 hr. later.

In spite of the filtration, the tank became cloudy on the second day after cleaning. A milliliter of the water was taken, successive dilutions made, and the higher dilutions cultured with a sea water and agar mixture (sterile aged sea water, agar, and a small amount of peptone and ferric phosphate). The number of resulting bacterial colonies were counted; the bacterial count of the original milliliter of aquarium water was computed at 212,000,000. Before the fish were placed in the aquarium the number of bacteria were probably less than 1,000,000 per ml.

Several smears were taken from eroded areas of dying manini. The dominant organism was a short, plump, gram-negative rod. It is not known whether this is the etiological agent of the disease or merely a secondary bacterium.

FOOD HABITS

Food of Larval Manini

Only three specimens of larval manini (6.6–7 mm. in total length) were available for an analysis of gut contents. Two of these fish were taken in a plankton net towed between 5:46 and 6:48 A.M. One was empty and the stomach of the other contained two appendicularian tunicates and a larval polychaete. The third fish

was captured between 3:20 and 4:25 A.M. and was empty.

The digestive tracts of 57 manini in the acronurus stage (see section on transformation), which were obtained at night light stations in the Hawaiian Islands, Line Islands, and Phoenix Islands, were examined in the same way. Forty-five were completely empty. Four contained one to seven tiny fish scales; one of these also contained a shrimp chela. A few crustacean appendages (mostly from shrimp) were found in three other fish, one of which had also eaten a barnacle metanauplius. The intestines of two fish had a small amount of unidentified chitinous remains. The crustacean appendages, metanauplius and fish scales were not free in the gut lumen but were caught between longitudinal folds of the gut. It seems evident that the acronuri do not feed at night.

Four acronuri of *Acanthurus* were obtained from the stomachs of skipjack (*Katsuwonus pelamis*) caught during the day in Hawaiian waters. Because of their being partially digested, I am not able to identify any of these acronuri to species; two, however, are too large to be manini. The stomachs and intestines of all four of these fish were filled with zooplankton. Shrimps and shrimp larvae were the principal food items. Also found were copepods, polychaetes, and the remains of larval fish.

Food of Transforming Manini

In an aquarium, manini in their first day of transformation from the acronurus to the juvenile stage were not observed to feed. The digestive tracts of 30 specimens, collected in tide pools during the morning of their first day of transformation and preserved immediately, were devoid of food material. Of 24 similar specimens collected in the afternoon, 19 were empty, but 5 had eaten small amounts of fine filamentous algae and leptopel (for a discussion of leptopel see Fox, Isaacs, and Corcoran, 1951). Second-day transforming manini were seen to feed on algae in an aquarium, though not frequently, and preserved specimens of the same transformation age all contained small amounts of algae and leptopel. During the remaining 2 or 3 days of transformation, feeding is progressively heavier.

Food of Juvenile and Adult Manini

The gut contents of juvenile and adult manini consist almost entirely of relatively fine filamentous algae. The teeth of the manini are close-set and denticulate on the margins (Randall, 1956 *b*: fig. 2a) like other species of *Acanthurus*. They are therefore well adapted for feeding on filaments of algae. Experiments on feeding in aquaria showed that the absence of coarser algae in the gut contents is due to limitation in the size and strength of the jaws and teeth. Only the smaller branches of coarser algae, like species of *Hypnea*, were eaten. Small juvenile manini only ate the fine end branches whereas larger fish ate progressively larger branches.

Inorganic sediment was rarely found in the stomach or intestines of manini. When branches of a delicate species of the red alga *Polysiphonia* were mixed with fine sand and offered to aquarium manini, the fish were most adept at picking out the filaments which projected above the sand. The stomach of the manini is thin-walled, similar to that figured and described by Breder and Clark (1947: 295, fig. 1) for *Acanthurus coeruleus*. It contrasts sharply with the thick-walled, gizzard-like stomachs of some species of *Acanthurus*. Unlike the manini, the latter species normally ingest large amounts of inorganic sediment with the algae upon which they feed.

In addition to avoiding the ingestion of inorganic debris, juvenile and adult manini do not seem to feed on animal material (although some species of *Acanthurus*, such as *A. xanthopterus*, will accept animal food readily). Even when hungry, manini declined to eat any of several kinds of animal food which were offered. In an aquarium the fish were very skillful in avoiding the intake of small crustaceans and small masses of tunicates or sponges which were in close proximity to the algae on which they were feeding. Occasional small animals are found in the gut contents, however. These are probably taken in accidentally. In shallow water at Coconut Island, Oahu, an adult manini was observed feeding on algae close to an egg mass of the damselfish, *Abudefduf abdominalis* (the guarding fish had been frightened away). When the manini reached the edge of the egg mass, it ceased to feed, swam over the mass, and began feeding on algae on the other side. Other fishes,

such as labrids and chaetodonts, feed greedily on the eggs when afforded the opportunity to do so by the absence of the guarding parent.

A study of the kinds of filamentous algae eaten by manini was undertaken. It was soon apparent that a great many different species of algae are eaten, and it is believed that at least a few filaments of virtually every filamentous alga in the Hawaiian area can ultimately be found in the gut of the manini if enough specimens are examined.

Dawson, Aleem, and Halstead (1955: 21), reporting on the gastrointestinal contents of 42 *Acanthurus triostegus triostegus* (size of specimens not given) from Palmyra, Line Islands, found 40 species of benthic algae and a number of species of diatoms of several genera. The number of algal species exceeded that previously known from Palmyra. Five species were most common in the stomachs: *Pterocladia* sp., *Sphacelaria furcigera*, *Lyngbya majuscula* (and/or *L. aestuarii*), *Bryopsis pennata*, and *Lophosiphonia* sp. The authors assumed that these were either dominant algae in the grazing grounds of the fish or were especially selected as food. It is the opinion of this author that these are the dominant algae, for (as is discussed below) the blue-green *Lyngbya majuscula* and another *Lyngbya* are not selected by this surgeonfish (at least not the Hawaiian subspecies).

A simple piece of apparatus was devised to test the preference by manini in Hawaii for various kinds of common filamentous algae. Ten tongue depressors were attached 2 cm. apart to a piece of wood. The free end of each depressor was notched and wrapped with a short piece of pliable galvanized steel wire. Different kinds of algae were fastened to the depressors by wrapping the wire ends around them. All 10 depressors with the assortment of algae attached were lowered simultaneously into the center of an aquarium in which juvenile manini, 26–30 mm. in standard length, were kept. When experiments were repeated, the position of the algae was always changed. If an alga was not eaten during the course of the experiment, it was left in the aquarium as long as it appeared edible to see if it would ultimately be devoured. The results are given in Table 3.

Many of the above algae were offered in a comparable manner to two adult manini, 123 and 128 mm. in standard length, with similar results.

Some species of algae not listed in the table were offered but not eaten; these, however, were probably too coarse for the juveniles. Included were two species of *Laurencia*, a species of *Gelidium*, a *Gracilaria*, and a species of *Dictyota*. A thallus of one of the species of *Laurencia* was placed in the aquarium with the adult manini. The small branches were eaten, leaving the major branches denuded.

Of the seven kinds of algae fine enough to be eaten but ignored in the preference experiments, one is a red (*Asparagopsis taxiformis*, well known for its high iodine content), one is brown (*Ectocarpus breviarticulatus*), and the rest are blue-greens. These were the only blue-greens tested.

Blue-green algae, some of which are the same species as those in the preference experiments, have been found in the stomachs and intestines of manini by the author (and, as noted, by Dawson, Aleem, and Halstead, 1955). This seems inconsistent with the results of the experiments. It was noted, however, that the blue-greens were never the exclusive food material, but were always mixed with large amounts of other algae, usually reds or greens.

In May, 1952, in water 10–20 ft. deep in Hanauma Bay, Oahu, the bottom was examined for the kinds of fine algae which reach a height of about 5 cm. or more. Three species this size were common: *Lyngbya majuscula*, *Asparagopsis taxiformis*, and *Plocamium sandvicense* (a red alga). Since surgeonfishes are abundant in the bay and at least two of the three algae are distasteful to one surgeonfish, the success of these algae might be associated with distasteful qualities.

Although there was little question from the observations that the manini requires no animal food, an experiment was designed to demonstrate that this species can subsist and grow on algae alone. A 23-gallon aquarium was divided into two compartments with a piece of plexiglass. Six manini, 26–31 mm. in standard length, were placed on each side and provided with cover in the form of several rocks grouped in the center of each compartment. The fish on one

TABLE 3

PREFERENCE FEEDING EXPERIMENTS ON JUVENILES OF *Acanthurus triostegus sandvicensis*

PREFERRED	COMPLETELY EATEN	SPARINGLY EATEN	NEVER EATEN
Polysiphonia sp.	*Hypnea* sp.	*Ectocarpus indicus*	*Lyngbya majuscula*
Enteromorpha sp.	*Hypnea* sp.	*Sphacelaria* sp.	*Lyngbya* sp.
	Lophosiphonia sp.	*Liagora* sp.	*Hormothamnion enteromorphoides*
	Ceramium sp.	*Jania* sp.	*Hydrocoleum cantharidosmum*
	Centroceras sp.	*Rosenvingia* sp.	*Calothrix confervicola*
	Gracilaria sp.	*Trichogloia* sp.	*Ectocarpus breviarticulatus*
	Rhizoclonium sp.	fixed diatoms (many species)	*Asparagopsis taxiformis*
	Dasya sp.		
	Enteromorpha sp.		
	Cladophora sp.		
	Grateloupia sp.		
	Herposiphonia sp.		
	Microdictyon setchellianum		

side were fed with a *Polysiphonia* and those on the other with an *Enteromorpha* (these were the algae upon which manini fed most vigorously in the preference experiments).

These algae are among the first macroscopic organisms to appear on the bottom of boats in harbor areas on Oahu. They are fast growing, especially the *Enteromorpha* which increased its length an average of 27 mm. per day on the eastern side of a boat during a period of 5 sunny days in April. If collected when the thalli first develop, they are almost devoid of animal life. A fresh mass of the *Enteromorpha* weighing 1.75 g. contained one ciliate, one nematode, one copepod, and a few epiphytic pennate diatoms and blue-green algal cells. Nevertheless the algae were thoroughly washed and picked over for animals before being fed to the fish. Fresh algae were added to the aquarium every day, and the previous day's algae removed. At the end of 2 weeks the fish were measured. Those fed on *Polysiphonia* grew an average of 2.9 mm.; those fed on *Enteromorpha* grew an average of 3.1 mm. During the 2-week interval the temperature in the aquarium was cool, varying from 23.0° to 23.8° C.

The preference experiments indicated that neither of these algae was eaten more readily than the other. Manini often alternated between feeding on the two. After 2 weeks of feeding on one of these, both kinds were added at the same time. It was immediately apparent that the fish had been conditioned to the alga on which they had been feeding. The "new" alga was untouched initially and only occasionally sampled an hour later. The preference was no longer obvious by the end of the day, however.

Manini feed almost constantly during the day, both in an aquarium and their natural habitat. They do not feed at night (see section on behavior).

The volume of algae consumed is large. Four juvenile manini, 28.5–45 mm. in standard length and weighing a total of 8.3 g., were fed a known mass of *Enteromorpha* in an aquarium early in the morning. The alga was weighed after firm squeezing followed by blotting on paper towels. At the end of the day the remaining fresh algal material was weighed in the same manner. Fresh *Enteromorpha* is grassy green and can easily be distinguished from fecal alga which is brownish or blackish green and tends to remain in pellets. The manini ate 10.8 g. of this alga one day and 8.8 g. the next. The same procedure was utilized for two adult fish, 123 and 128 mm. in standard length and 83.2 and 94.5 g. in weight, respectively. These two fish ate 27.4 g. of *Enteromorpha* in one day. The second day 16.3 g. of *Polysiphonia* was consumed. After being unfed during the morning of the third day, they ate 25.2 g. of *Enteromorpha* during the remaining 6 hr. of the day. Although these two adult manini in the above experiment were well adapted to aquarium life (they were reared to this size as captive fish in a pond of the Hawaii Marine Laboratory at Coconut Island and were

maintained in the aquarium 12 days before the experiments), it was observed that they did not feed as frequently as adult manini in the natural environment (whereas the juveniles did). The feeding by the adults was readily interrupted by the approach of an observer. Usually only one fish fed at a time, and since the larger one dominated the smaller and held it in a corner of the aquarium for much of the day, the former consumed the major part of the algae. Yet both fish disdained to feed at all when placed in separate aquaria.

Stimulus to Feeding

The following simple experiments and observations were conducted in order to ascertain what sensory mechanism or mechanisms are utilized by the herbivorous manini in finding food.

The juice from several grams of *Enteromorpha* was squeezed into an aquarium in which two adult manini had been starved for a day. This was unfiltered and colored the water green when first dropped into the aquarium. No response was observed, even when some of the green color was seen to diffuse in the immediate vicinity of the nose and mouth of the fish. A similar experiment was performed on several juvenile manini with *Polysiphonia*, again with no visible response. By contrast, when an extract of *Polysiphonia* was dropped into an aquarium containing a half-grown *Abudefduf abdominalis* (omnivorous in food habits), this fish swam to the surface where the extract clouded the water red and snapped its jaws erratically in this region.

Some *Enteromorpha* and *Polysiphonia* was boiled to the extent that the algae began to disintegrate and lost most of their color. This was placed in the aquarium with fresh algae of the same kinds. Although juvenile manini fed initially on the fresh algae, the boiled algae was ultimately completely eaten.

Some maroon rayon fiber similar in texture and color to *Polysiphonia* was placed in an aquarium with juvenile manini. It was taken into the mouth temporarily by several of the fish. When the rayon was soaked in extract of the *Polysiphonia*, it was not taken in with greater frequency.

When recently voided fecal *Enteromorpha* was placed at the surface of the aquarium in the same manner that fresh algae were offered, most of the manini approached it, hesitated, and swam away. One juvenile drew a small amount into its mouth but hastily ejected it. When defecated filaments were added together with fresh filaments of *Enteromorpha*, only the fresh alga was eaten. In the absence of fresh algae, fecal algae which have remained in the aquarium for several hours may be ingested. The results of offering the blue-green algae listed in Table 3 were similar to that just described for recently voided fecal algae. The blue-greens were approached, but rarely taken into the mouth.

The nasal organs of a 26 mm. manini were cauterized with a hot wire. Five min. after this, the fish was offered some *Polysiphonia*. It fed on this with the same "enthusiasm" as previously.

Another juvenile fish was blinded by wiping a crystal of potassium hydroxide over the eye (other fish were blinded with silver nitrate crystals but these invariably died within 36 hr.). After contact with the potassium hydroxide, the surface of the eye became opaque white; this fish survived more than 3 days. It moved slowly about the bottom, occasionally swimming by chance into a mass of *Polysiphonia*. It never swam directly to the algae. When the algae touched the mouth it was often eaten. By the third day when the aimless movements carried the fish into a mass of algae, it fed voraciously.

It is concluded from the above that vision alone is needed to locate algal food, and the olfactory sense functions in feeding only to avoid unsavory material. Chemoreceptor organs in the mouth and possibly tactile organs as well also seem to be associated with the acceptance of algae and the rejection of material which is not utilized as food. The tendency to feed more on one alga than another develops after the algae have been sampled.

DIGESTION

In the work on nutrition of marine animals much effort has been expended on the analysis of mode of feeding and stomach contents, whereas little has been applied to the important

aspect of the digestion of these animals. Yonge (1931) pointed out that information as to what an animal may collect and pass into its alimentary system may or may not indicate the true food of the animal. Thus, a study of digestion should logically accompany an investigation of food habits. The author is especially indebted to P. B. van Weel of the University of Hawaii for his counsel in the following research on digestion.

Morphology of the Digestive Tract

The similarity of the stomach of the manini to that of *Acanthurus coeruleus* as described by Breder and Clark (1947) has been mentioned. The manini stomach is elongate, and divisible into cardiac and pyloric portions. The cardiac part has prominent, longitudinal, irregularly scalloped folds on the inner surface; the pyloric part is smooth. A cross-section of the pyloric part showed the muscle tunic to be about the same width as the mucosa. There are few multicellular glands. The stomach wall is less than 0.1 mm. thick except posteriorly near the pylorus where it is slightly thicker than 1 mm.

Just posterior to the pylorus are five pyloric caeca. Several authors (including Yonge, 1931) have claimed that the pyloric caeca has taken over the role of the pancreas in certain teleosts, the latter organ supposedly being absent in these fishes. Dawes (1929) found that the pyloric caeca of the plaice (*Pleuronectes platessa*) had the same structure as the intestine with which it was in free communication, and Rahimullah (1945) came to the same conclusion after examination of 119 species in 50 different families of fishes. The structure of the pyloric caeca of the manini is consistent with the findings of Dawes and Rahimullah. The bile duct opens into the base of one of the pyloric caeca.

The pancreas of the manini was difficult to find. It was finally located in the form of two to four (usually three) small, round glands buff in color lying in the mesentery alongside the bile duct near its junction with the liver.

The intestine of the manini is long, in keeping with the well-founded biological principle that herbivorous animals have lengthy intestines. The length of the alimentary tract of large adult manini is nearly six times the standard length of the fish. Most of this length is attributable to the intestine which is complexly folded within the body cavity. The length of the alimentary tract of small juvenile manini is only slightly greater than three times the standard length of the fish. The increase of the alimentary tract relative to standard length is shown in the graph of Figure 3. With increasing size the volume of a body requiring nutriment increases faster than the intestinal surface if the rate of growth of the two is equal. A disproportionate elongation of the intestine is necessary to keep the area of absorptive surface adequate to the needs of the body. Hiatt (1947: 254, 257) noted a striking increase in the relative growth of the intestine of the herbivorous milkfish (*Chanos chanos*) between 90 and 115 mm. standard length. The ratio of intestinal length to standard

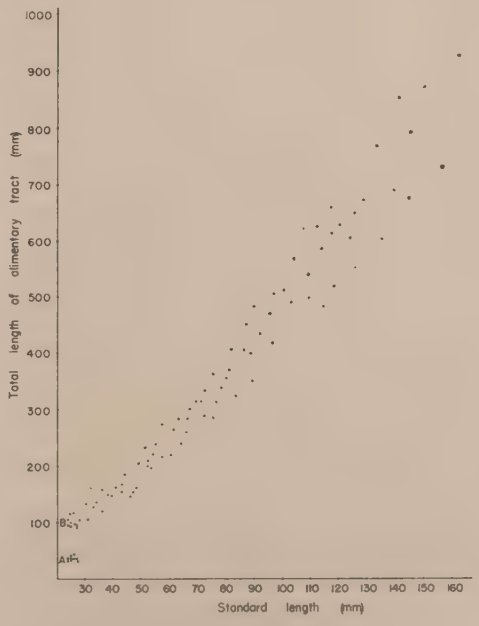

FIG. 3. Change in length of the alimentary tract of *Acanthurus triostegus sandvicensis* with increase in standard length. The group of points at *A* represents the length of the tract of 10 specimens (24–27.5 mm.) in the acronurus stage which were taken at a night light offshore. The points at *B* represent 10 tide-pool specimens (24–28 mm.) which have just completed transformation from the acronurus to the juvenile stage.

length increased from 3.5:1 to 7.2:1. He demonstrated a correlation between the increase in length of intestine and the substantial rise in the amount of larger algal types in the stomach contents and the reduced number of smaller food items like diatoms and blue-green algae. No sudden change-over in the size of algal food which is eaten by the manini occurs at any size range once transformation has taken place, and the curve of alimentary tract length plotted against standard length is without sharp inflection. The great increase of the gut length which occurs when manini transform from the acronurus to the juvenile state is discussed in the section on transformation.

The inner surface of the intestine is covered with small villi. The wall of the intestine is thin, its average thickness, not including any villi, is about 0.05 mm. The mucosa is about three to four times as broad as the muscle tunic.

Time for Algae to Pass through Gut

In order to find out the time which is required for algae to pass through the entire alimentary canal of the manini, two adults, 123 and 128 mm. in standard length, were fed only the red alga, *Polysiphonia*, for a period of 2 weeks. The tank was cleaned and the diet switched to the green alga, *Enteromorpha*. Two hr. and 25 min. later the fish were observed defecating the green alga. They had already deposited some on the bottom of the aquarium, so the following day the experiment was repeated by switching back to the red alga. In 2 hr. and 4 min. the first of the red alga was voided. In view of the great length of the digestive tract, this short interval of time seems extraordinary. However, when considered in the light of the nearly constant diurnal feeding and the volume of algae consumed, it becomes more understandable.

The same procedure was repeated for small juvenile manini, ranging from 26 to 30 mm. in standard length. The *Polysiphonia* passed through their alimentary tracts in 1 hr. and 45 min.

At night, when feeding ceases and the fish enter a state of torpor, the gut is not completely emptied in 2 hours or so. One 39 mm. manini, for example, which was caught at 1:15 A.M. still had a small amount of algae in the stomach and in the last 26 mm. of the intestine. The total length of the alimentary tract of this specimen was 177 mm. A 123 mm. manini was observed to defecate *Enteromorpha* 14 hours after it last fed on this alga.

Digestion of Algae

Lefèvre (1940) has shown that different kinds of algae resist digestion by gastrointestinal juices of fishes in various degrees. Although supporting growth of manini as well or better than the *Polysiphonia*, the *Enteromorpha* appeared less digested. Filaments of this alga were found to be only slightly altered after their rapid passage through the gut. The cellulose cell walls seemed unaffected, and the cell contents still in place. The only discernible change in most cells was the breaking up of the parietal grassy green chloroplast to brownish-green granular clumps. A visual comparison was made of the quantity of starch granules in the *Enteromorpha* cells following staining of fresh and fecal algae, and no obvious differences were apparent. Clearly, more work is needed to elucidate the problem of algal nutrition of the manini. The possibility that the pectic sheath material of algae is digested should be investigated.

Digestive Enzymes

No reference was found in the literature to any assay of the digestive enzymes of a strictly herbivorous fish. From work on herbivorous animals other than fishes the generalization can be made that proteinase is less active in herbivorous forms than in carnivores and amylase more active. There are indications that this is true for fishes, at least for amylase. Kenyon (1925) compared starch digestion in the carp (*Cyprinus carpio*) which, though omnivorous, eats large amounts of algae, and the carnivorous pike (*Esox lucius*). He wrote, "... the carp, which is largely a vegetarian, possesses amylase in tremendous amounts in the hepatopancreas and to a less extent throughout the intestinal mucosa. The pickerel, on the contrary, ... possesses only a negligible quantity of amylase, having little in the pancreas, esophagus, intestine, and practically none in the stomach." Vonk (1927)

wrote that the carp has more than 1,000 times the amount of amylase in the pancreas than that of the carnivorous pike or shark. Schlottke (1939) found amylase in large quantities in the carp, whereas the predaceous rainbow trout (*Trutta iridea*) and perch (*Perca fluviatilis*) evidently produced almost no amylase. Data comparing the activity of proteinase and lipase of omnivorous and carnivorous fishes are meager and conflicting.

In view of the importance of the hydrogen ion concentration to enzyme activity, the pH of the contents of various parts of the digestive system of the manini was determined. The measurements of pH were made with a Beckman pH meter on six adult fish which averaged 120 mm. in standard length. The results, expressed in the ranges of pH found, are given in Table 4.

TABLE 4

pH OF ORGANS OF THE DIGESTIVE SYSTEM OF *Acanthurus triostegus sandvicensis*

ORGAN	RANGE OF pH
Stomach	6.3–7.7
Duodenum	7.7–9.1
Intestine	8.0–9.1
Gall bladder	6.2–6.4

The variation of pH within any one organ appears to be correlated with the degree of fullness of the organ. The low pH values were found in the organs when they were filled with algae. Hydrochloric acid secretion in a stomach in which food is present is a probable explanation for the greater acidity at this time. Babkin and Bowie (1928) found a variation in pH of the duodenum of the killifish (*Fundulus heteroclitus*) similar to that shown above for the manini. These authors also noted that low values of pH were obtained when the duodenum contained food. They attributed this to the discharge of bile to the organ when filled with food.

Extracts for the enzyme study were consistently prepared from the stomach (both cardiac and pyloric portions combined), pancreas, pyloric caeca, duodenum, and intestine of adult manini which were killed immediately before the removal of these organs. Because of the excessive thinness of the gut wall, it was very difficult to separate the mucosa from the muscle layers; therefore extracts were made of entire organs or linear parts of organs. All portions of the digestive tube to be extracted were first washed with sea water to remove food material. Tissues were ground in mortar and pestle with calcareous sand. This sand had previously been cleaned by repeated washings with water, boiling with 3 per cent potassium hydroxide and then for a short while with 2 per cent hydrochloric acid. In view of MacKay's (1929) report that 30 per cent alcohol yielded the most active amylase from the eel pout (*Zoarces anguillaris*), this agent was also used to extract amylase in the present study. Lipase extracts were made in 40 per cent glycerol and protease extracts in 50 per cent glycerol. Extraction was carried out in a refrigerator for a period of 24 hr.

Digestion by amylase and lipase proved to be rapid at room temperature (26°–27° C.); thus no incubation was necessary in experiments with these enzymes. Digest tubes with protease were incubated at 36° C.

Buffer solutions used in the digestion experiments were based on the mixtures of Clark and Lubs (Hawk and Bergeim, 1942: 24). Bacterial action was prevented by the addition of several drops of toluol to the extract and digest test tubes.

The substrate for amylase experiments was 1 per cent starch solution. To each test tube containing 1 ml. of extract of the digestive organs 5 ml. of starch solution and 1 ml. of buffer of pH 6.8 were added. For each tissue there was a control tube identical with the experimental digest tube except for the previous boiling of the extract to inactivate all enzymes.

The progress of digestion was followed by removing small amounts of fluid from the digest tubes and testing with Lugol's solution. The changes in the solution from deep blue-black through purple, red, yellow, and finally colorless indicated a breakdown of the starch at least to achroodextrine. Ultimately all of the tubes were colorless, thus disclosing starch digestion by the pancreas, pyloric caeca, duodenum, intestine, and stomach. The experiment was repeated three times with sections of the digestive tract vigorously washed to minimize the possibility of enzyme from another source being adsorbed on the epithelial surface of the organ being tested.

Again, there was a definite amylase reaction from each organ.

The positive results seem unusual in view of the fact that most vertebrates (except for mammals which secrete ptyalin in their saliva) break down starch initially with pancreatic amylase and complete the process with intestinal maltase. These results on the manini seem less dubious, however, in the light of the finding by Kenyon of amylase throughout the whole gut of the carp (although it was considered to lack a true stomach) and by the detection of stomach and duodenal amylase in *Zoarces* by MacKay. Also Babkin and Bowie found amylase in the intestine of the killifish. They were certain it was not adsorbed pancreatic amylase, for they were unable to observe any proteolytic action in the same extract.

Pancreatic amylase of the manini is nearly 20 times more powerful per unit of tissue than the amylase from other organs, while that from the stomach was weakest (attempts were made to obtain extracts from equal amounts of glandular tissue of the organs under comparison).

The pH optimum of the amylase, as determined by color change with Lugol's solution and the micro method of Linderstrøm-Lang (Linderstrøm-Lang and Holter, 1933), is 6.7.

The Schoorl method was utilized to test for the presence of maltase in the stomach, pyloric caeca, pancreas, duodenum, and intestine of the manini. One per cent maltose solution served as the substrate. Trials for all organs were run at pH 7.0 and 7.2 and incubated at 30° and 35° C. for periods up to 12 hr., but results were consistently negative.

The method of Michaelis and Rona (see van Weel, 1937: 245) was used in lipase experiments. Tri-n-butyrin solution was used for the substrate. Digestion occurred rapidly in all the organs tested. It was evident that the pancreas produced the most lipase and the stomach the least, although the difference was not as marked as with amylase. The pyloric caeca showed the greatest lipase activity of the remaining organs. Difference between the duodenum and the rest of the intestine was not discernible. The pH optimum determined for pyloric caeca lipase of the manini is 7.2.

Detection of protein digestion was based on the formaldehyde titration of Sörensen (Jordan, 1927). The substrate was a 3 per cent colloidal solution of gelatin.

In initial experiments protease was found in the pancreas, pyloric caeca, duodenum, and intestine, but not in the stomach. In none of the organs was the proteolytic activity strong. The pH optimum of pancreatic protease is 8.4.

Because of the acidic reaction in the stomach of the manini and the knowledge that protease in this organ can vary widely from individual to individual depending on the state of hunger of the animal (Schlottke, 1939), further effort was expended to localize this enzyme in the stomach. Extract of high concentration (prepared from trituration in 5 ml. of 50 per cent glycerol of three adult manini stomachs, two of which contained considerable algae) finally gave positive results. One ml. of this concentrated extract (thus containing the extractable enzyme from three-fifths of a stomach) at pH 6.0 yielded acid equivalent to 0.2 ml. of 0.015 normal sodium hydroxide after 4 hr. of incubation.

A piece of the very thin covering (one cell layer thick) from one of the internal rings of an onion was peeled off and placed in a glass stender and covered with the fluid from the intestine of an adult manini. The onion skin was examined after 24 and 48 hr. periods, but no digestion of the cellulose cell walls occurred. The experiment was repeated with fluid from the intestine of another adult specimen, again with negative results. Thus there appears to be no cellulase-secreting micro-organisms in the intestine of the manini.

It is concluded that the results of the enzyme study of the manini are consistent with the generalization previously made concerning the digestive enzymes of herbivorous animals except for the absence of cellulase.

REPRODUCTION

Sex Ratio

No sexual dimorphism in external morphology was noted; therefore gonad examination was necessary for sex determination. The gonads lie in the ventroposterior part of the body cavity. No difficulty was experienced in distinguishing an ovary from a testis macroscopically except with immature fish. The ovaries are pinkish

cream in color, smooth, slightly compressed laterally, and closely applied to one another in the mid-line. The testes are white, strongly compressed, irregularly lobular, and in contact with each other only ventrally.

It was apparent from sampling the catch of trap fishermen throughout the year that approximately twice as many male manini as females were taken. In the month of May, 1953, 291 trap-caught adult fish were sexed; 68 per cent of these were males. The traps in which the fish are caught are unbaited. A typical trap consists of a rectangular framework (about 2 × 5 × 6 ft.) of steel rod covered with chicken wire. A cone of chicken wire with a narrow slitlike opening extends inward from one end to about the center. Some trap fishermen prefer to leave one or two butterfly fish or other fishes of little economic value in the trap in the belief that other fishes will be more prone to enter. If male manini are more gregarious or less cautious or more migratory than females, they would be caught in traps with greater frequency than females; therefore sex ratios ascertained from trapped fish should be viewed with caution.

The sex was determined for 221 adult manini from islands in the tropical Pacific in the collections of the U. S. National Museum, the Bernice P. Bishop Museum, and the University of Hawaii. Most of these specimens were taken with rotenone, seine, and throw net. These collective techniques are less selective than traps and the sample would seem to be a better indication of true sex ratio. Of the 221 fish, 134 (60.7 per cent) are females. A chi-square value of 10 results from testing whether 134 females could be obtained from 221 fish randomly sampled from a population in which the true sex ratio is 50-50. This high chi-square has a probability greater than 0.01 that a sample more extreme than the above could be drawn by chance from a half male, half female population; thus the hypothesis of a 50-50 sex ratio is rejected.

It is nevertheless possible that the apparent predominance of female manini is spurious. Most of the 221 specimens were taken in inshore areas. The traps from which 198 males of 291 manini were caught were set in from 30 to 90 ft. of water. Perhaps females are more abundant in shallower water and the males predominate over deeper parts of the reef. More collections are needed to clarify this problem.

The museum collections of manini offer some slight evidence for differential schooling by sexes. One sample of 7 manini from the outer reef at Eniwetok Atoll, Marshall Islands, is entirely male; another of 12 fish from the lagoon of Kwajalein Atoll in the Marshalls is wholly female.

Spawning Cycle

The manini in the Hawaiian Islands has a distinct spawning season. This has been ascertained by the examination of the gonads of adult fish and collections of young throughout the year. In more equatorial areas, on the contrary, the spawning of this surgeonfish appears to be year-round. There is also evidence both in Hawaii and the Gilbert Islands that the pattern of the incoming young correlates with the lunar cycle. This is attributed to a lunar effect on spawning by adults.

From October 31, 1952, to October 27, 1953, a total of 137 female and 156 male manini 100 mm. or more in standard length were purchased in 41 samples from a fisherman for the purpose of examination of the gonads. The fish were caught in traps set in depths of 30–90 ft. from Kewalo Basin to Koko Head, Oahu. The length of the gonad on each side of the body cavity of each specimen was measured and the average length recorded. This average measurement was divided by the standard length and the quotient multiplied by 100. The range and mean of the resulting percentages are presented in Table 5.

Although the monthly samples are small, it is nevertheless apparent that the gonads of both sexes undergo a significant change in relative length during the year. They are largest in late winter and spring and smallest in late summer and early fall. The change which takes place in the ovary in late July and August is more complex than would be indicated from a mere shortening. The ovary (presumably following the last spawning) is red, flattened, and has a large lumen. Residual eggs are gradually absorbed (eggs being absorbed appear as amorphous masses of variable size), and the entire organ regresses to small size and a translucent gray color. There is

TABLE 5

GONAD LENGTH AS A PERCENTAGE OF STANDARD LENGTH BY MONTH, 1952–53

MONTH	NO. OF FEMALES	GONAD LENGTH / STANDARD LENGTH × 100		NO. OF MALES	GONAD LENGTH / STANDARD LENGTH × 100	
		Range	Mean		Range	Mean
Oct.	15	7.1–18.2	9.4	14	5.6–22.8	9.4
Nov.	2	7.7–14.4	11.1	12	8.3–22.8	15.5
Dec.	4	12.3–25.0	14.3	11	10.0–22.1	15.9
Jan.	9	8.6–25.6	13.5	10	8.4–25.0	17.9
Feb.	15	14.1–25.6	18.2	31	20.4–34.5	28.6
Mar.	19	13.5–32.2	19.3	25	12.8–35.7	28.6
Apr.	17	10.4–26.4	17.3	7	22.8–38.5	29.4
May	6	9.8–23.3	16.2	5	18.2–31.2	25.6
Jun.	9	7.0–34.5	13.5	5	15.0–25.1	20.0
Jul.	10	7.5–17.6	9.6	9	8.1–26.3	14.9
Aug.	9	5.5–11.9	7.2	8	6.5–23.8	13.2
Sept.	22	5.0–14.5	8.4	19	7.6–17.3	9.0

variation in the time when this occurs in individual fish, hence the ultimate small size is not apparent when the values of a number of fish are averaged by month. The ovary of a 145 mm. female taken on August 7, for example, was only 8 mm. in length.

The gonad measurement data are more significant in indicating a definite spawning season of the manini when coupled with the results of sampling the young throughout the year. Transforming and small juvenile specimens were absent from inshore areas during the fall and early winter months of 1952–53. Extensive observation and collecting were undertaken during the winter and early fall to ascertain the time of the first and last arrivals of acronuri from the pelagic realm. The first transforming young were sighted on February 14; the last of the season straggled in on October 6. In 1954 the first young were discovered on February 9. During my absence from the state, my wife and Philip Helfrich made an effort to determine the date of the last influx of young in 1954. In October only a single small juvenile was collected; it was taken on the 9th of the month and measures 27.5 mm. in standard length. It is completely transformed; therefore it had been in the tide-pool zone for about 5–8 days (see sections on transformation and growth). The first transforming young of the 1955 season were observed on January 27.

The time between the finding of the first ripe female of the season and the first incoming young and the time between the last ripe female and the last incoming young constitute estimates of the duration of larval life. The last ripe female of the 1952–53 season detected in the sampling program (see Table 6) was found on July 21. The first ripe female of the 1953–54 season was found on December 1. The last ripe females of this season were observed in the July 16 sample. The three estimates of the duration of larval life from the above data are 71, 78, and 83 days. Admittedly such estimates are subject to considerable error. Their average, 77 days, approximates 2½ months.

In order to obtain quantitative data on the recruitment of young to inshore areas of Oahu, a single large tide pool at Diamond Head (Fig. 4) was chosen for repeated collections of manini. This pool is nearly isolated at low tide. An overhanging ledge on the left (east) side of the pool provides excellent cover for the small fish. Pools as large as this but lacking such cover contain fewer manini. Each week at low tide the narrow outlet to the sea was occluded with a net, and all the manini in the pool were poisoned with rotenone. A total of 157 manini less than 30 mm. in standard length were taken from the pool during the season (Fig. 5). Nineteen specimens 30 mm. or longer were collected; these are presumed to have been in the tide-pool zone for more than one week (see section on growth) and hence are probably migrants from adjacent pools. They are not included in

FIG. 4. Tide pool at low tide at Diamond Head, Oahu, from which the collections of *Acanthurus triostegus sandvicensis* of Figure 5 were made.

the graph of Figure 5. The poison stations on August 13 and August 21 were interrupted by unexpected large waves, and no fish were collected. However, on these two dates manini were counted in the pool before rotenone was added and these numbers are indicated by the dotted line on the graph. Probably there were at least twice as many small manini in the pool as are recorded, for most of the fish take cover rapidly when an observer approaches and are hidden before they can be counted. The sample of 36 fish taken on September 12, on the other hand, may be higher relative to the overall number of tide-pool young at this time than it should be. Observation of other pools at Diamond Head and along the shore of the Ala Wai Yacht Basin prior to, during, and after September 12 failed to disclose any striking influx of young. Nevertheless, the number of incoming young in September is high and this month should be included with the period May to August as indicating large tide-pool recruitment on Oahu.

Although this sampling from a single pool is not sufficiently great to be correlated closely with the similarly inadequate samples of adult gonads, the May to September recruitment seems to correspond roughly to the greater degree of gonad development from February to June.

In more equatorial waters the *A. triostegus triostegus* appears to spawn throughout the year. The 221 adult fish used for the sex ratio determination were collected mostly from the Mari-

FIG. 5. Weekly collections of *Acanthurus triostegus sandvicensis* made in 1953 from a single tide pool at Diamond Head, Oahu. Only specimens 30 mm. or less are recorded. Times of new moon are indicated by black circles.

ana, Marshall, Gilbert, Line, and Phoenix islands—groups in which the sea surface temperature exceeds 80° F. (26.7° C.) the year-round and where the annual variation in sea temperature is slight (based on Hydrographic Office, 225). Examination of these fish revealed ripe gonads in every month of the year. Specimens of transforming or small juvenile manini collected every month of the year from these areas were found in museums.

It is, therefore, puzzling that the seasonal spawning in the Hawaiian Islands seems to be associated with the colder part of the year. The mean monthly sea surface temperatures around Oahu (1945–55) vary from about 75° to 81° F. in an average year (Leipper and Anderson, 1950; Hydrographic Office, 280.) The temperature of the warm part of the year is essentially the same as that of the lower latitudes where spawning is year-round. The initial enlargement of the gonads in Hawaii coincides with the time of decreasing sea surface temperature in the fall. The marked increase in tide pool recruitment in May appears to correspond to increased spawning in February–March, the time of coldest water temperature.

If temperature or some other factor such as change in length of day is the cause of interrupted spawning in Hawaii, then manini in a region of the South Pacific Ocean with comparable latitude should spawn seasonally and 6 months out of phase from Hawaiian manini. Unfortunately no data are available from southerly island groups such as the Australs or southernmost Tuamotus where sea temperatures closely approximate those in Hawaii.

Small juvenile and transforming *A. triostegus triostegus* were taken or observed by the author in every month of the year (1956–57) in the Society Islands (where temperatures range from about 77.5° to 81° F., or 25.2°–27.2° C.). The manini is not as abundant in the Society Islands as in Hawaii, and the insignificant tide in the former island group made it difficult to find numerous individuals isolated in tide pools. Collections were insufficient to demonstrate any possible variation in reproductive activity during the year.

A cyclic fluctuation in the abundance of incoming young within the spawning season in the Hawaiian Islands is apparent from Figure 5. The peaks of these fluctuations line up roughly with the time of new moon. This correlation is more evident from the extensive collections made from various tide pools plotted in Figure 6. The large mode of May 10 centered on a standard length of 26 mm. contains 54 transforming fish. The time of new moon was May 13. The large mode of April 17 is centered on a standard length of 27 mm. and contains 10 transforming manini. New moon in April occurred on the 13th day.

Tester and Takata (1953: 36, fig. 14) have demonstrated a similar lunar periodicity in the appearance of young aholehole (*Kuhlia sandvicensis*) in tide pools on Oahu.

If the periodicity in the influx of manini acronuri is a function of some lunar effect on the young and not on the spawning adults, then the peak fluctuations of incoming acronuri in areas of different temperature, and hence different rates of development (see development section), should still correlate with the time of new moon. A sample of 108 small manini collected with rotenone by the author from tide pools at Onotoa Atoll, Gilbert Islands, on August 21, 1951 (Fig. 7), demonstrates that this is not the case. Onotoa is located in the central Pacific (1° 47′ S., 175° 32′ E.); the monthly mean sea surface temperatures vary only slightly from 82.5° F. (28.6° C.) throughout the year. Full moon occurred on August 16 (new moon on August 2). The large mode of Figure 7 centered on about 23 mm. standard length includes only 9 transforming specimens which average 23.5 mm. in standard length. Thus the peak influx of the fish comprising this mode is estimated at 4 or 5 days prior to August 21. This large Onotoa sample, therefore, is about 11 days out of phase with the lunar cycle of incoming young on Oahu (where the mean monthly sea surface temperatures vary from 75° to 76.5° F., or 23.9°–24.5° C., from January to April).

In order to obtain direct evidence of lunar spawning by adult manini and to more sharply delimit the spawning season, the entire catch of two trap fishermen was examined for ripe fish on 62 days from June 19, 1953, to September 3, 1954. The catch was usually brought to port alive. The fishermen would not permit the open-

ing of the body cavity without purchasing the fish, but they allowed pressure to be applied to the abdomen of each manini.

When running ripe males (the sperm of which were motile) were discovered in samples taken throughout the year, it was realized that the limits of the spawning season could not be defined by the development of the male gonad. As would be expected from Table 5, however, there were more ripe males during the spawning season than outside of it. Of 745 adult manini (since trap caught, an estimated two-thirds were

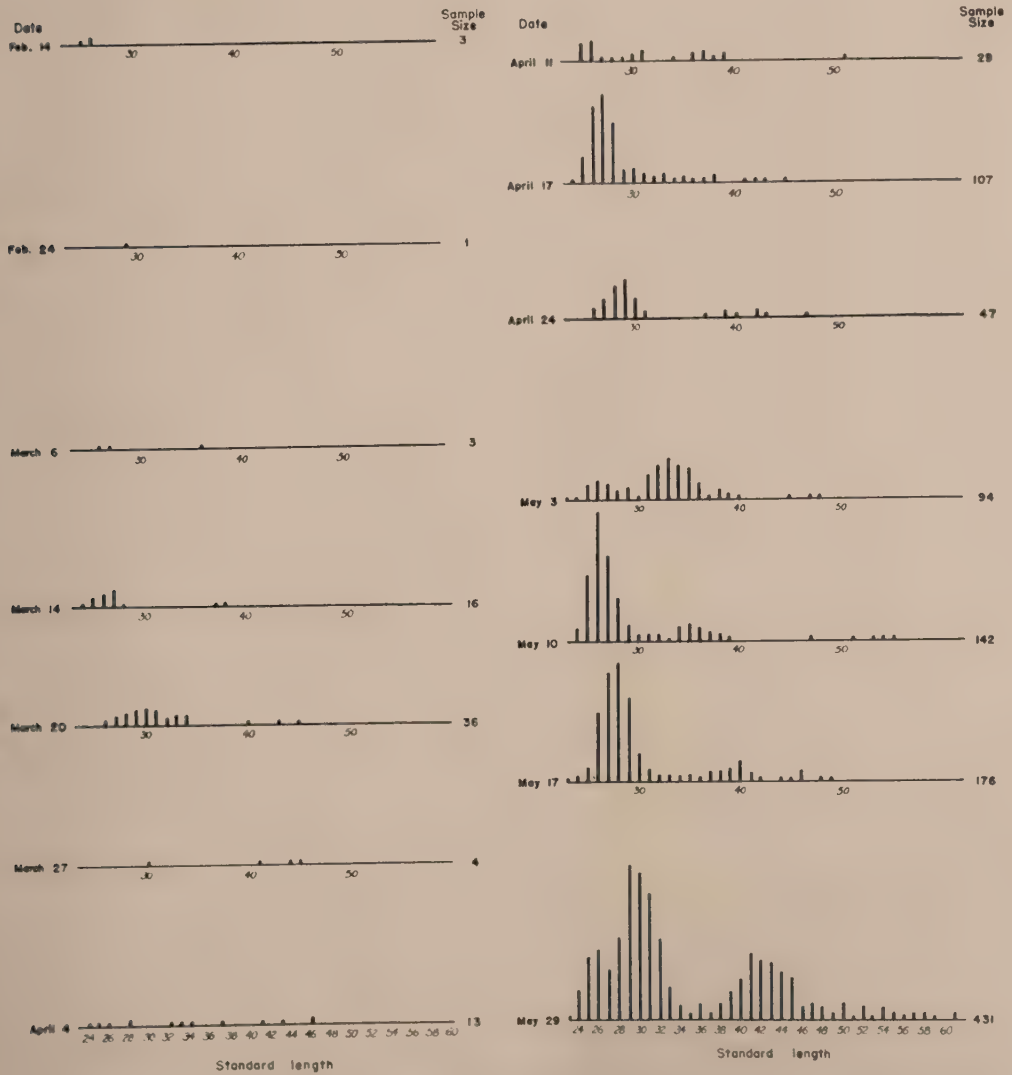

FIG. 6. Tide-pool collections of *Acanthurus triostegus sandvicensis* from Ala Wai Yacht Basin to Makapuu Point, Oahu, 1953. Vertical distance between horizontal lines is proportional to number of days between samples.

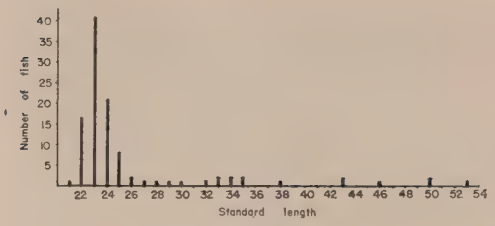

FIG. 7. Collection of *Acanthurus triostegus triostegus* from tide pools of the outer reef flat, Onotoa Atoll, Gilbert Islands, August 21, 1951. Sample size, 108. Nine specimens 21–25 mm. in standard length are transforming from the acronurus to the juvenile stage; the rest are juveniles. Time of full moon, Aug. 16.

males) examined from September to November, 11.8 per cent were running ripe males; 23 per cent of 2,419 adult manini (an estimated two-thirds of which were males) examined from February to June were running ripe males. No variation in degree of ripeness was apparent within the period of a single month. It should be emphasized, however, that no sharp dividing line exists between the condition of a running ripe male and one that is not quite running ripe, and these data tend to be subjective in this regard.

Ripe females are more easily distinguished. Fertilizable eggs of female manini are about 0.7 mm. in diameter, prefectly transparent, and are released when the abdomen is only slightly squeezed. The data on ripe females in terms of the lunar month are summarized in Table 6. As may be seen in this table, the number of fish examined on the different days ranged from 9 to 252. This variability is entirely due to variation in the catches. Also there is no regular sequence in time when the catches were examined. Scheduled sampling was not possible largely because of the great dependence of trap fishing on hydrographic conditions. In addition to being impeded by rough seas, trap fishermen are restricted by turbid water (traps are not found by surface markers but by observing through a glass-bottom box). Although the fishermen ordinarily tend their traps twice a week, they may be held in port several weeks by dirty water. A successful sampling day was also contingent on being at Kewalo Basin when the catches were unloaded. Frequently the fishermen came in unexpectedly early and their fish were promptly taken to market. Market fish were not sampled because catches of previous days may accumulate and the date when fish are caught is usually not available.

A total of 43 running ripe females were found. Thirty-nine of these were taken from 12 days before to 2 days after the full moon and only 4 from the remaining half of the lunar month. A total of 2,552 adult manini were examined in the former period, however, and only 1,311 in the latter; therefore a correction is necessary for this discrepancy in sample size. A corrected figure of 7.8 ripe females instead of 4 is the proper number to compare with 39. Nevertheless, it still remains obvious that a significantly greater number of ripe females, and hence probably greater spawning, occurs before and shortly after full moon than before and shortly after new moon. Since at least a few acronuri of all sizes (see section on transformation) enter tide pools at all times during the month and not totally in one portion thereof, some spawning might be expected throughout the month.

One sample of adult manini was examined on July 29, 1955. There were 7 running ripe female fish among the 57 manini comprising the sample. Full moon occurred on August 3.

It is not known whether spawning by the same fish takes place every month. There is evidence, however, that individual fish spawn more than once a season. The long season in itself suggests repeated spawning. If large ripe females were found at the start and progressively smaller ones throughout the rest of the season, a long spawning season might be indicated with each individual spawning only once, but no obvious trend in size of the 43 ripe females was apparent. The first ripe female of the season, which was found December 1, measured 138 mm. in standard length. The last (July 21) was also large, 140 mm. The shortest found were 106 mm. (March 13) and 101 mm. (July 14). More convincing is the failure to see any completely spent ovaries in fish during the spawning season and the observation of spent and regressing ovaries in all of the females at the end of the season. The strongest evidence concerns the finding in ripe females of a second mode of egg size

TABLE 6

RESULTS OF SAMPLING FOR RIPE FEMALE *Acanthurus triostegus sandvicensis* DURING THE SPAWNING SEASON

DATE	DAYS TO NEAREST FULL MOON		NO. OF FISH EXAMINED	NO. OF RIPE FEMALES
	Before	After		
Jun. 19, 1953	8		40	1
21	6		9	1
Jul. 14	12		114	1
17	9		45	0
21	5		14	1
Dec. 1		11	141	1
8	12		126	1
22		2	139	0
29		9	96	0
Jan. 8, 1954	11		149	1
12	7		21	1
24		5	26	0
Feb. 5	12		30	0
9	8		30	0
12	5		41	1
Mar. 2		13	31	0
12	7		144	6
30		11	44	0
Apr. 2		13	87	0
6	12		198	0
9	9		121	0
13	5		111	0
16	2		193	7
20		2	132	6
23		5	76	0
27		9	151	0
May 1		13	88	0
6	11		83	1
11	6		226	3
14	3		149	2
18		1	18	0
21		4	204	1
25		8	159	0
28		11	103	2
Jun. 11	5		42	2
18		2	97	0
22		6	29	0
25		9	49	0
Jul. 2		14	27	0
9	7		28	0
16	0	0	252	4

about half the size of the ripe egg mode, coupled with the knowledge that this mode is not retained in the ovary through the summer months. This mode of intermediate-size eggs appears to be a persistent unit within the ovary of all mature female manini during and 1–2 months before the spawning season, suggesting that it constitutes a static mass of eggs from which modes of ripe eggs may develop periodically. It is not known whether this mass is built up constantly or at short intervals from the huge reserve of minute primordial eggs which is present in the ovary of every adult female regardless of the time of year (and is here not considered as a mode of egg size).

The procedure for the determination of egg diameters was as follows: Tiny sections were snipped from various parts of an ovary (which was previously preserved in 8 per cent formaldehyde solution) and placed in water in a Syra-

cuse watch glass; the eggs were teased from the ovarian tissue with dissecting needles; the egg diameters were then measured with an ocular micrometer at × 24. Many of the eggs were oblong, probably because of unequal pressure of adjacent eggs at the time of preservation. Instead of measuring the greatest or least diameter, the eggs were moved into position by moving, but not turning, the watch glass on the micrometer image which remained fixed in a horizontal position. This method reduces the possibility of bias but has the disadvantage of increasing the spread of modal groups on graphs of egg diameter measurements.

Graphs of the egg diameters of ovaries from 9 adult female manini are shown in Figure 8. These graphs do not show any definite sequence.

Graph *C*, of a 15 mm. ovary from a female taken on January 2, for example, portrays the egg diameter pattern of an immature but maturing ovary. No graphs were made of egg diameters of ovaries from adult fish taken in August because the fish have only primordial eggs about 1 to 3 micrometer units (.035 to .105 mm.) in diameter except for those in which larger eggs are being resorbed. The relative size of the group of primordial eggs was estimated only for the ovary graphically illustrated in *A*.

Except for the immature gonad of *C*, all of the ovaries display a mode of egg size centered on about 10 to 12 micrometer units in diameter. This prompted the examination of samples of eggs from 54 ovaries taken from female fish throughout the year (more, however, from mature females during the spawning season). Sufficient eggs from each ovary were measured to determine the presence of obvious modal groups. A definite mode at from 9 to 12 micrometer units in egg diameter occurred in 46 of the ovaries, 15 to 35 mm. in length, from fish caught from September 15 to July 21. The median of this mode of 38 of these fish appears to lie between 10 and 12 micrometer units. In the ovaries of 8 fish, the median lies between about 9 and 10 units. One of these fish is the only running ripe female of the 54 examined. The remaining eight fish lack an intermediate-size mode; all of these have small gonads. No specimens were found among the 54 females with a group of eggs which have an average diameter greater than 12 units but less than the 20 to 21 unit average size of ripe eggs. Also, when checking for ripe females among the 3,863 adult manini of Table 6, the eggs pressed from the body cavity of few, if any, of these were of a size between that of the usual mode and the ripe egg mode. It is expected that such a size would be extruded with moderate pressure on the abdomen of the fish, since eggs of about 10 micrometer units (0.35 mm.) are frequently forced out. Eggs as large or nearly as large as completely ripe eggs, but in which small opaque white regions were visible, were seen almost as often as the ripe, perfectly transparent eggs. These can not be fertilized, and females containing them were not considered among the ripe fish previously discussed. The failure to find a mode of eggs intermediate in size between about 10 and 20 micrometer units suggests that eggs maturing from the former modal group (the eggs of which are opaque) do so rapidly. Perhaps the enlargement is essentially a process of hydration.

In view of the finding of so few female manini with fertilizable eggs during the season, it is further believed that the ripe eggs are not retained very long in the ovary. Admittedly a change in behavior of ripe females might influence the frequency with which they enter traps (a possible cessation of feeding, however, would not seem to affect the rate of entry, for the traps, as mentioned, are unbaited). Also, confinement in the traps might affect the maturation of the eggs.

If, however, it is assumed that ripe eggs are held within a female no longer than 1 day on the average, and 43 ripe female fish is the number that would be present in truly random samples of the population equal in size to the ones taken, then the small number of ripe females actually constitutes an indication that every adult female spawns once each lunar month. Since the lunar month is 29.53 days, only about one-thirtieth of the females sampled from the population during the spawning season would be ripe. As mentioned, 3,863 adult manini were examined during the 1953–54 season, an estimated one-third of which, or 1,288, were females. One-thirtieth of 1,288 is 43, precisely the number of ripe females which were found.

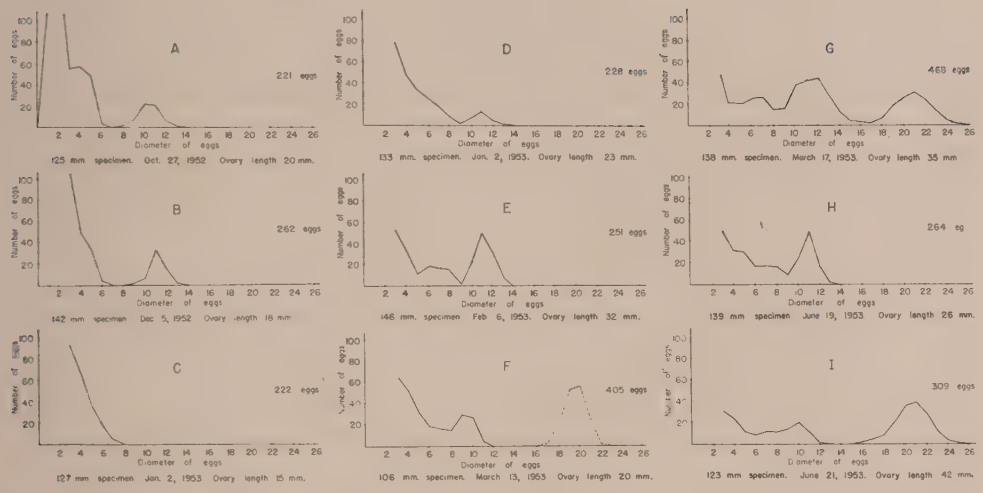
A
221 eggs
125 mm. specimen. Oct. 27, 1952 Ovary length 20 mm.
B
262 eggs
142 mm. specimen Dec. 5, 1952 Ovary length 18 mm.
C
222 eggs
127 mm. specimen Jan. 2, 1953 Ovary length 15 mm.
D
228 eggs
133 mm. specimen. Jan. 2, 1953. Ovary length 23 mm.
E
251 eggs
146 mm. specimen Feb. 6, 1953. Ovary length 32 mm.
F
405 eggs
106 mm. specimen. March 13, 1953 Ovary length 20 mm.
G
468 eggs
138 mm. specimen. March 17, 1953. Ovary length 35 mm
H
264 eg
139 mm specimen June 19, 1953 Ovary length 26 mm.
I
309 eggs
123 mm specimen. June 21, 1953. Ovary length 42 mm.
Number of eggs
Diameter of eggs

FIG. 8. Egg diameters of *Acanthurus triostegus sandvicensis* plotted as units of an ocular micrometer. Scale: 1 unit = .035 mm. Except for *A*, where the number of eggs of 1 and 2 micrometer units were estimated at 1,575 and 627, respectively, no eggs less than 3 micrometer units were measured.

Spawning

The spawning of *A. triostegus sandvicensis* was never witnessed, in spite of considerable observation both at night and during the day and at various times of the month. On June 12, 1957 (full moon) *A. triostegus triostegus* was observed to spawn at the atoll of Tikahau in the Tuamotu Archipelago at dusk (5:30 P.M.). It is believed that the failure to see the Hawaiian manini spawn was due to the lack of effort at dusk. The following account of the reproduction of the Tuamotu manini is probably similar to that for the Hawaiian subspecies.

Several hundred spawning *A. triostegus triostegus* were observed in the pass at a depth of about 25 ft. Many of the fish displayed a marked color change. The black bars on the side of the body were much broader (but the added width on either side of each bar was not as intense a black as the center); the median fins were dark, almost black, except for the middle of the caudal which remained pale; the dark median band on the forehead was prominent. Five fish of this color pattern were speared (when pursued, an individual dark-colored manini resumed normal coloration within about 10 sec.); all 5 were ripe males. As many as 4 or 5 of these more melanistic fish were repeatedly observed chasing single fish which were unchanged in color pattern. Two of the normally colored manini were speared. One was a running ripe female and the other a female with opaque eggs which were squeezed from the body only with considerable pressure; thus not all females in the spawning aggregation are capable of reproduction at the same time, which is in keeping with the finding of running ripe female manini in Hawaii along with fish with maturing ova on many different days of the lunar month (Table 6). Actual spawning took place among small groups of fish within the large school which became increasingly active and suddenly darted upward about 8 ft. above the rest. Eggs and sperm were released at the apex of this movement. Swimming was more rapid as the apex was approached. The fish dispersed as they swam back down to mingle with the school. At times two or more small groups joined together in the first few feet of the upward movement, resulting in a dozen or more fish in a compact mass at the top. The attempts to spear individual fish broke up the one large aggregation into several lesser ones in which spawning took place infrequently.

Two other surgeonfishes, *Ctenochaetus striatus* (Quoy and Gaimard) and *Zebrasoma scopas* (Cuvier), were observed to spawn in the Society Islands. Like the manini, they exhibited the sudden upward rush prior to spawning. Possibly the release of eggs and sperm is facilitated by the expansion of the airbladder from the decreasing pressure caused by the upward swimming movement. Like the manini in the pass at Tikahau, both of these surgeonfishes were spawning in a region of strong current to the open sea.

Size and Age at Maturity

The smallest running ripe female seen by me was 101 mm. in standard length. The smallest running ripe male was 97 mm. in standard length. These are probably near the minimum lengths for mature manini around Oahu.

Ten manini were reared in a large concrete tank of the Hawaii Marine Laboratory at Coconut Island from a size of 25 to 27 mm. in standard length beginning on March 17, 1953 (see detailed discussion in section on growth). Two fish of this group, an 89.5 mm. male and a 99 mm. female were killed for gonad study on July 24, 1953. The female had an immature gonad, clear gray in color, and only 5 mm. in length. The ova were minute, only 0.015 to 0.03 mm. in diameter. The male was detected because it was possible to strip a small amount of milt from it, although considerable pressure on the abdomen was necessary. The sperm, however, were not motile. Its testis was slender and 6 mm. long. Subsequent examination of the gonads of the remaining 8 captive manini was not made because of arrested growth of these fish following inadvertent destruction of the algal food supply when seining the tank.

There appears to be considerable variability in the size at maturity. This variability is apparent when the 101 mm. ripe female manini is contrasted with the 127 mm. immature specimen whose egg diameters are graphically illustrated in Figure 8, *C*.

It is doubtful that any female manini spawn during the season in which they first arrived as acronuri in tide pools. Even if the 99 mm. female, which was killed on July 24 following its growth in captivity from a small juvenile on March 17, had been among the first few arrivals to shallow water of the season (middle February), another month of growth and gonad development would hardly seem sufficient to bring a tiny immature ovary such as that seen in this specimen on July 24 to full maturity before the season ended. It also seems unlikely that males will spawn within their first season following recruitment to tide pools.

It seems probable, from a knowledge of growth (see growth section) and the approximate size at maturity, that some manini will spawn in the season following their first sojourn as juveniles in inshore waters. This would be very likely for the first young of the season; the last young of the season would not seem to have sufficient time to develop to sexual maturity before the following spawning season ended. Such a situation would probably increase the variation in the size of the fish at maturity.

Fecundity

Fecundity is defined as the total number of ripe eggs produced by a female in 1 year. It is impossible to ascertain this for fish like the manini which may spawn more than once a year, when the number of spawnings per year is unknown. Therefore only the number of eggs released at one spawning can be determined. This was attempted for only a single manini.

The fish chosen for the egg count was the 123 mm. June 21 specimen, the egg diameters of which are plotted in Figure 8, *I*. This fish had a very large ovary (42 mm. in length) which contained a high percentage of large eggs. These eggs were not completely transparent, and none appeared to have been expelled by the fish. The ovary was vigorously shaken in the vial in which it had been preserved with a minimum of fluid. The agitation released nearly all of the near-ripe eggs from ovarian tissue and placed them in uniform suspension. The contents of the vial were then placed in a graduated cylinder, allowed to settle, and the small amount of excess fluid poured off. A sample of the ovarian mass was drawn off. After its removal, the volume was computed as 26 per cent of the total. All of the large eggs in this sample were counted in successive lots placed in a Petri dish under a binocular dissecting microscope. The sample

contained 10,814 large eggs. The total number of large eggs in this ovary was therefore estimated at 40,000.

DEVELOPMENT

Fertilization of the eggs of the manini was effected artificially, using live fish obtained from trap fishermen. As previously discussed, ripe female fish are detected by the release of perfectly transparent eggs following gentle pressure on the abdomen. The milt of running ripe male manini oozes even more readily from the fish than the eggs of ripe females; however, it was found that eggs could be fertilized with sperm from males in which definite squeezing was necessary to bring forth the milt. Eggs were fertilized both by stripping ripe males and females concurrently into sea water with genital apertures in close proximity and by stripping them separately into different containers and subsequently mixing the contents.

The fertilized eggs are spherical, 0.66 to 0.70 mm. in diameter, and contain a single oil globule which is 0.165 mm. in diameter. If suspended in sea water, the eggs slowly rise to the surface. They exhibit no stickiness and do not adhere to objects or one another, although they often lie in single-layered groups at the surface.

Unfertilized (but fertile) eggs are also less dense than sea water. No difference in diameter of unfertilized and fertilized eggs could be detected.

Developing eggs were placed in large finger bowls, an aerated battery jar, and an aerated 20 gal. aquarium. In all of these containers heavy mortality of the larvae ensued because of settling to the bottom (probably through contact with bacteria there and not to any physical injury). Efforts were made to keep the larvae in suspension by placing a fine-mesh false bottom in a battery jar and locating a bubbling aerator stone beneath a hole at one side and by installing a paddle which was slowly turned by an electric motor. Although these methods reduced the amount of settling, mortality was still high and only a few larvae survived to an age of 5 days or more, when oriented swimming began.

In view of the difficulty of rearing larvae in rigid containers, a new approach was tried for the manini. A fine-mesh bag, 1.2 m. on a side, with cork along the top was floated in the Ala Wai Yacht Basin. Fertilized eggs were placed within and allowed to develop. Approximately the same mortality resulted, however.

Once the manini larvae were capable of oriented swimming, they were maintained as easily in unaerated finger bowls as any other way. At this time the critical factor became the supply of food.

The principal stages in early development from 0 hr. to 5 days 22 hr. are shown in Figures 9–12. The drawings of eggs before hatching were made shortly after placing the eggs in 10 per cent acetic acid, a treatment which makes the blastodisc opaque white and the cleavage pattern more visible. The ages given are based on an average development temperature of 24° C. During development the temperature was 24° C. ± about 1°.

Although temperature control was not sufficiently precise to permit an assay of the change of rate of development with changing temperature, it was obvious from the rearing of one batch of eggs at 26° C. ± 1°, that temperature has a marked effect on the rate. The stage attained at 28 hr. 40 min. at 24° C. was reached in about 22 hr. 30 min. at 26° C. The 42-hr. stage at 24° C. was reached in about 32 hr. at 26° C.

No attempt was made to determine the temperature limits within which normal development of the manini is possible. Due to proximity of a light bulb, the temperature of one finger bowl in which 37-hr. larvae were developing was inadvertently raised in a short time to 29.3° C. None of the larvae died at this time.

The blastodisc of the fertilized egg is 0.4 mm. long. One hour after fertilization the first cleavage becomes apparent. At the end of the second hour 8 cells are present, and after 4 hr. about 55 cells can be counted in the blastodisc.

At the age of 6 hr. gastrulation is under way. Epiboly is evident from the thin layer with a wavy edge which is extending downward over the yolk. The cells are very small and are not shown in the drawing of this and subsequent stages.

At 14 hr. the primitive streak is apparent and the cephalic region of the embryo well developed. By about 18 hr. epiboly is complete, and

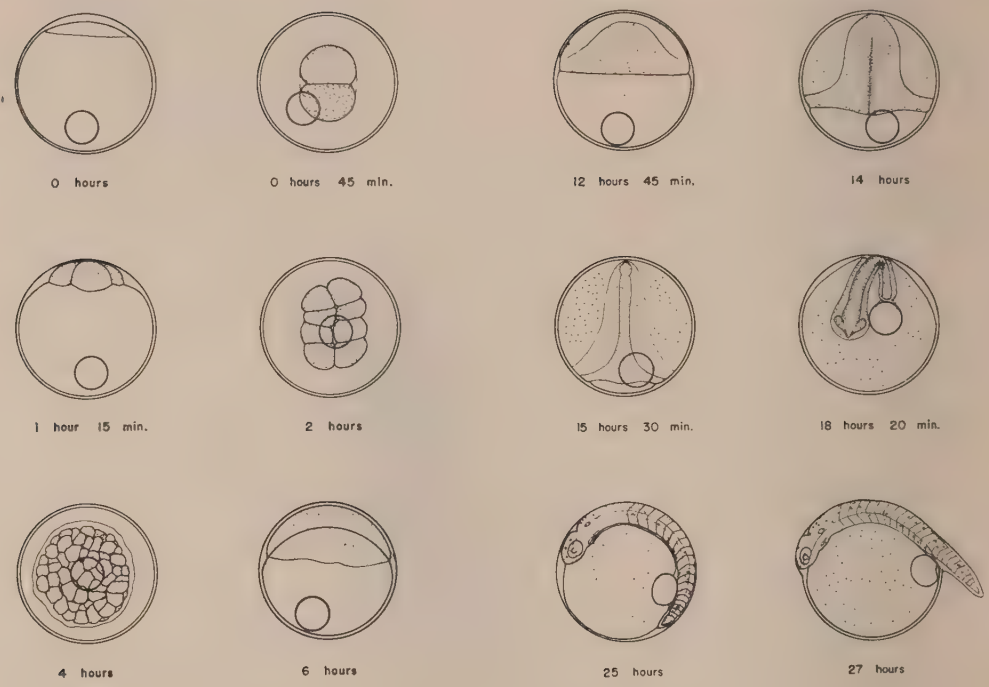

FIG. 9. Developing eggs of *Acanthurus triostegus sandvicensis*. Egg diameter, 0.67 mm.

the yolk plug is visible as a small clear area near the oil globule.

By 15 hr. 30 min. the body of the embryo is beginning to take form and elongate over the yolk. At 18 hr. 20 min. 9 somites can be counted and the optic vesicles are prominent. At 21 hr. 45 min. 12 somites are present. At 25 hr. 19 or 20 somites are visible; the lens of the eye is just starting to form; the auditory vesicle is present and the statoliths are just forming; 24 tiny melanophores can be seen in lateral view on the body; twitching movements are common; the heart contains blood although it has not yet started to beat.

Hatching occurs at the age of about 26 hr. At 27 hr. the larva is nearly 1.7 mm. in length and has 22 somites; the heart is still quiescent. At 31 hr. the heart was observed to beat; occasional short random swimming movements occur by rapid vibration of the posterior half of the body.

At the age of 42 hr. the amount of yolk is reduced to about half of what is present in the 31-hr. larva. As the yolk is used up, the larvae show a progressive tendency to sink. Larvae of 39 hr. still float head down at the surface, but 42.5-hr. fish have begun to settle. The rate of settling of 1 larva at this age was measured at 1.2 cm. per min. The average rate of 3 44.5-hr. larvae was 1.67 cm. per min., and the average rate of 4 47-hr. larvae was 1.88 cm. per min. One 71-hr. larva sank at the rate of 8.2 cm. per min.

At 42.5 hr. some larvae were already settling out at the bottom of the aquarium. Most, however, maintained their level by swimming movements. 300 such movements were observed in an aquarium which contained several hundred larvae. Of these movements, 227 resulted in the larvae being in a higher position in the tank; the rest were sideways or downward. Movements which consisted only of a twitch were not

counted. The ability to overcome settling is more efficient than 227 movements with an upward component out of 300 would indicate. Since the head is directed downward, swimming is initiated in this direction. In most cases the fish soon turn sharply and swim upward. If the interval of movement is short, there may be insufficient time to end up higher than the starting position, but usually the fish is not significantly lower. The interval between movements is highly variable, but it averages about 1 min. The distance traveled by the larvae per movement is usually less than 40 mm. One, however, stopped 110 mm. above its starting position.

In addition to geotaxic responses, swimming movements were made to avoid contact with an approaching object. This was first observed in 42-hr. larvae; however, it may be operative several hours earlier. The eyes do not seem sufficiently well developed to be functional at this early age, and a glass rod is detected as easily as an opaque one; thus vision would not seem to be the sense responsible for perception of an approaching object. Before such an object touches a larva, and sometimes when it is as far as 2 or 3 cm. away, a fleeing movement is initiated. A second stimulus soon after the flight reaction will usually result in a second swimming movement. Contact with the bottom due to slow settling usually will not result in a swimming movement. Regular movements occur at about the same rate when the fish are on the bottom. They may enable a larva to become suspended again, but usually only for a short time.

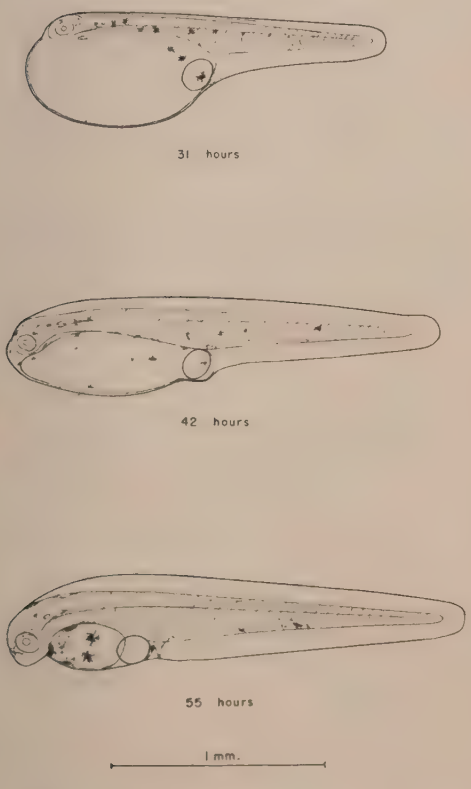

FIG. 10. Early larval stages of *Acanthurus triostegus sandvicensis*.

Areas of white pigment are beginning to form on the ventral part of the seventh and fifteenth somites of the 42-hr. larva. These are large and conspicuous in the 54-hr. larva, and lesser white areas may be seen on the ventral part of the body anterior to the seventh somite and on the head. At this stage the intestine has just formed and the yolk mass is small. The melanophores over the yolk are large and dendritic.

Figure 11 consists of microphotographs of living 75-hr. and 4-day 1-hr. larvae with the light transmitted through the little fish. The dorsal fin fold is a prominent feature at 75 hr. (it was first noticed in the 66-hr. larva), and the pectoral fins are developing. Dark pigment around the eye is evident. The 4-day 1-hr. larva was observed to sink less rapidly than prior stages. One larva of this age sank 2.5 cm. per min. and another less than 1 cm. per min. The 4-day 12-hr. larva remained suspended head down in the water, thus indicating that the air bladder was functional. The jaws were observed to move in the 4-day 5-hr. larva (Fig. 12).

Although still tending to float head downward, the 5-day larva is capable of normal oriented swimming. The pectoral fins can be fluttered rapidly, and the eyes move. The yolk is completely gone and the oil globule half resorbed. By 5 days 12 hr., the 3 individuals which survived to this age appeared to be feeding by short darting movements. Material from a culture of marine ciliates was added to the finger bowl in which these 3 larvae were kept. One

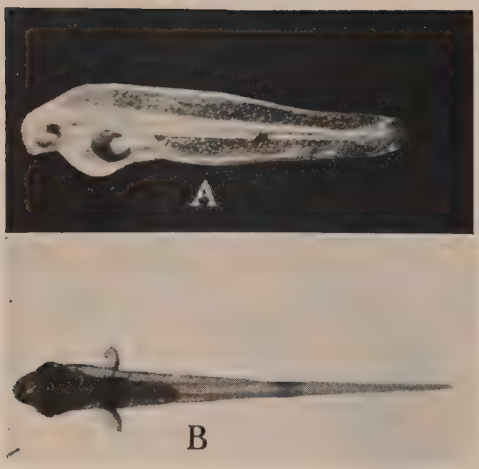

FIG. 11. Microphotographs of larval *Acanthurus triostegus sandvicensis*. *A*, Side view of 75-hr. larva; length 2.3 mm. *B*, Four-day 1-hr. larva in top view; length, 2.5 mm.

died after 5 days 17 hr. of development. Food was present in the intestine of this specimen. The second member of this trio was observed to be dying at the age of 5 days 22 hr. It was removed from the finger bowl for the lower drawing of Figure 12. As may be seen in this drawing, no trace of the oil globule remains, the intestine is convoluted, the liver is prominent, and the gall bladder is visible. The two large white areas on the body have disappeared. The total length is nearly 2.7 mm. The last larva survived to the age of 6 days 12 hr. It grew very little in the last 24 hr., presumably because of insufficient or inadequate food.

Three of the 11 acanthurid lavae from the POFI plankton collections (see p. 216) were identified as manini by dorsal and anal fin-ray counts. They are 6.6–7.0 mm. in total length. Figure 13 is a drawing of the 6.6 mm. specimen (standard length 5.3 mm.). The teeth are not visible without opening the jaws. They are simple canines. The total length of the anterior upper teeth is contained 3.7 times in the diameter of the pupil of the eye.

Specimens smaller than this one could not be identified by ray counts, for the soft rays are not fully formed. A 4.2 mm. specimen could not be positively identified at this time to genus, for only the first three dorsal spines are sufficiently developed to distinguish them from soft rays; however this specimen is about intermediate in structure to the 5-day 22-hr. larva and the 6.6 mm. larva (though is closer to the latter). Its body depth is contained 1.8 times in the total length, and the relative length of the second dorsal, second anal, and pelvic spines is about two-thirds as great as that of the 6.6 mm. larva. The caudal fin is about half formed, the urostyle extending to the posterior part of the incipient fin.

A 4.3 mm. specimen was the only acanthurid found in the POFI larval fish collections which were taken with a 6-ft. modified Isaacs-Kidd trawl, except for an 18 mm. *Zebrasoma veliferum* (Randall, 1955*c*: fig. 3). It was captured near the surface off Kahuku, Oahu. Although a little longer than the specimen just mentioned, it is in a slightly earlier stage of development and is probably a different species. If a large series of specimens were available instead of just a few it might be possible to identify the various stages of the manini to the size where fin-ray counts alone can provide definite identification.

No postlarval specimens of *Acanthurus* greater than 8.7 mm. in length but smaller than the acronurus form were found in any of the POFI collections or museum collections. As previously discussed, specimens larger than about 9 mm.

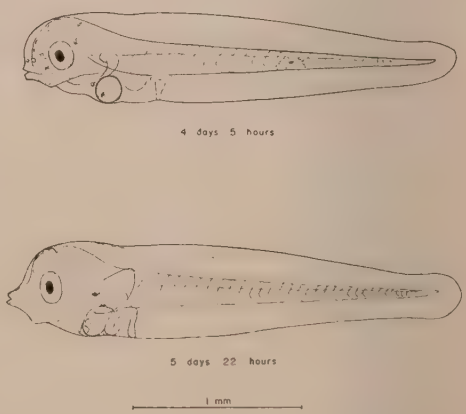

FIG. 12. Larval stages of *Acanthurus triostegus sandvicensis*.

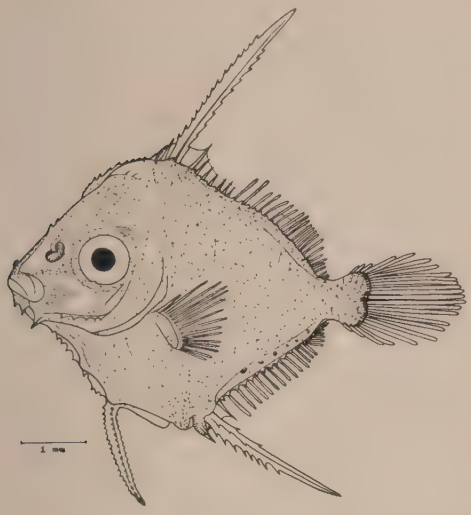

FIG. 13. Postlarval *Acanthurus triostegus sandvicensis* taken in a 1 m. plankton net at a depth of 50 m. at 22° 38′ N., 157° 11′ W.

in length probably elude a meter plankton net. The presence of the 18 mm. *Zebrasoma veliferum* in the collections from the 6-ft. trawl suggests that this would be a better means of catching larger larval acanthurids. The failure to take more acanthurids in the trawl is probably due to the great distance from land of most of the trawl tows.

The only records of the early stages of *Acanthurus* found in the literature are two postlarval *A. coeruleus* identified by Lütken (1880: pl. 5) and an unidentified 7 mm. postlarval *Acanthurus* in Weber (1913: fig. 70). Spartà (1928) reported on a long, ribbon-like, transparent, pelagic egg mass of an unknown teleost fish from the Mediterranean. The early developmental stages which he succeeded in rearing from this egg mass are remarkably similar to those of the manini. Spartà concluded that the eggs were from a species as yet unrecorded from the Mediterranean.

TRANSFORMATION

The late postlarval form of the genus *Acanthurus* is characteristic in morphology, yet identifiable to family Acanthuridae by the presence of the caudal spine and a diagnostic number of fin rays. Originally this stage was placed in a genus by itself, *Acronurus*. The name acronurus has persisted as a common name for this stage, reminiscent of the leptocephalus of eels.

Acronuri are commonly taken at night-light stations. Sixty-two manini acronuri and many of other species collected at night lights and by night-light traps offshore in the vicinity of the Hawaiian, Line, and Phoenix islands were examined. No specimens in night-light collections were found which were smaller than the acronurus stage. It is inferred, therefore, that a positive phototaxis develops in the acronurus. The lack of food material in the gut of night-light specimens (see p. 223) indicates that the presence of numerous small planktonic animals around a light at night is not the attractant. Once transformation to the juvenile stage has begun, the positive phototaxis disappears.

Figure 14 is a photograph of a live specimen of the manini acronurus taken shortly after its arrival in a tide pool. The characteristic transparent scaleless body with silvery abdomen and head (except snout and nape), disk-like form, and vertical striae on the body are apparent. The vertical bars are just beginning to form.

The acronurus comes into inshore regions

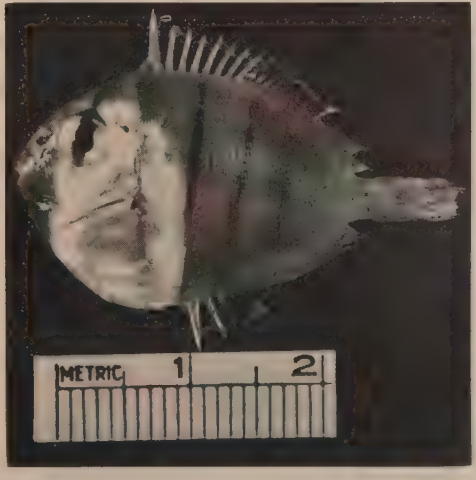

FIG. 14. The acronurus of *Acanthurus triostegus sandvicensis*. Captured at dawn in a tide pool at Diamond Head, Oahu, and photographed alive.

only at night. New arrivals to inshore areas have been collected all through the night, some as early as 9 P.M. and others just before dawn. Most of the night collecting was done at low tide. It is not known whether the influx of acronuri is greater at one phase of the tide than another.

On two occasions before midnight at low tide in ankle-deep water along the shore of the Ala Wai Yacht Basin near the entrance to the Ala Wai Canal, manini acronuri were observed just coming into the area from deeper water. They did not passively float into the shallow zone but swam in rapidly. One crossed several times through the beam of a head lamp before it was caught. If it is assumed that these fish were in deep water beyond the breaker zone before nightfall, then they must have actively swum into the harbor area and not been carried in by any tidal currents. Prior to low tide, tidal currents would be flowing out of the yacht basin and not into it. This is contrary to the belief of Breder (1949*a*: 296) that acronuri of *Acanthurus hepatus* (= *A. chirurgus*) are carried into shallow water by "vagaries of current."

Breder reported observing transforming specimens of *A. chirurgus* at sizes from 23 to 29 mm., and found juveniles from 10 to 20 mm. in length in tide pools at Bimini. He inferred that late postlarval surgeonfish reach a certain size in the plankton at which transformation is possible but continue to grow. The size at transformation is dependent on the size of the acronurus when it reaches shallow water. Breder's explanation of the cause of this variability in transformation size is plausible, although a range of at least 10 to 29 mm. for one species seems high.

From April 11 to October 4, 1953, a total of 175 manini were found in shallow water on Oahu in their first day of transformation to the juvenile stage. Their standard lengths (measured to nearest 0.5 mm.) ranged from 22 to 29.5 mm. Even this would seem, a priori, to be more variation in length than would be expected from mere growth variation alone in the pelagic habitat. A small amount of this variability in Hawaii is due to the change in temperature at which development occurs during the season (Fig. 16 and discussion below), but considerable variation can be seen in the transformation size of acronuri taken within the period of a single month, and therefore cannot be attributed to seasonal temperature differences.

The fluctuation in abundance of incoming young manini has provided an opportunity to test Breder's hypothesis. If the variation in size at transformation were entirely due to normal variation in growth, then no difference should be apparent in the size of manini which are transforming during the time of the month when a big influx is occurring and the period when the recruitment to inshore areas is minimal. If, however, more extremes in size were found when the recruitment is at a minimum, it could be assumed that growth of the manini occurs in the plankton after transformation is possible and that members of a modal group which arrive inshore and transform earlier or later than the majority of the group will be smaller and larger, respectively.

In Figure 15 the lengths of the 116 transforming manini which were collected within the period 5 days before to 5 days after peak tide-pool recruitment (taken as 2 days before the time of new moon; see p. 234 and Fig. 6) are compared to the lengths of the remaining manini caught outside this period. Clearly, a higher percentage of extremes in length at transformation occur during the part of the month when the number of acronuri entering shallow water to transform is low.

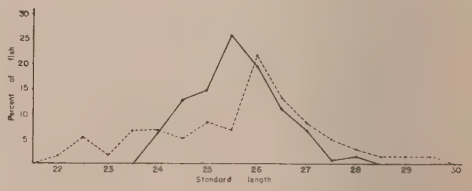

FIG. 15. Size of *Acanthurus triostegus sandvicensis* during the first day of transformation from the acronurus to the juvenile stage. The solid line represents the percent of fish at the designated standard lengths from samples taken 5 days before to 5 days after peak tide pool recruitment (3 days before new moon). The dotted line represents the percent of fish from samples taken during the rest of the lunar month. The solid line is based on a total of 116 fish and the dotted line on 59 fish. Samples include all of the first day transforming manini which were collected from Apr. 11 to Oct. 4, 1953.

If spawning of the manini occurred only during a definite small segment of the month, the dotted curve of Figure 15 would probably be bimodal, one of the modes centered on a low and the other on a high standard length. Since, however, spawning appears to occur throughout the month and merely more prominently in one part than another, most of the young resulting from fertilization at a time of low spawning intensity will come into shoal areas at a more nearly average size during the interval between periods of high tide-pool recruitment and obscure the postulated bimodal effect. The extremes in size of this small group would enter the tide-pool zone at the time of peak recruitment; thus, if the samples were large enough, both curves would extend to the same limits.

The maintenance of populations of manini around small islands such as Johnston Island, the shores of which are constantly swept by a strong current in one direction, would seem to demand some means on the part of the developing pelagic larvae to remain close to land. The presence of small eddy systems around an island might afford the explanation, provided the larvae can stay within such systems. The apparent differentiation of the manini at Johnston Island (Randall, 1956*b*: table 2) and also of *Ctenochaetus strigosus* (Randall, 1955*d*) would seem to rule out the possibility that the Johnston Island populations are derived primarily from fishes which spawn in the Hawaiian Islands proper.

While examining museum specimens of transforming manini from many localities, a definite variation in size with locality was noticed. The standard lengths of the available early transforming museum specimens were measured. These lengths (except those of Hawaiian Islands specimens, already presented graphically) are given in Table 7 with localities and with collection dates when known.

A correlation exists between the size of manini at transformation and the temperature of the water of the locality. The warmest region listed in the table is the East Indies where the mean monthly sea surface temperature may reach 85° F. (29.4° C.) (Hydrographic Office, 225). Here we find the smallest size at transformation, 20–21 mm. Small standard lengths, averaging less than 23.5 mm., are found in other warm areas such as the Palau, Marianas, Gilbert, and Marshall islands (all regions where the mean monthly sea surface temperature exceeds 81° F., or 27.3° C., during the entire year), and southern India in January, and Okinawa in September. Transforming specimens from regions where the temperature is 80° F. (26.6° C.) or less throughout the year or during the month of the date of collection are large, 24 mm. or greater in standard length. These areas include Durban (S. Africa), New South Wales, Ningpo (China), Hawaiian Islands, Mangareva, the Marquesas Islands (in August), and Clarion Island. The large transformation size of the specimens from the Phoenix Islands, where the sea surface temperature exceeds 82° F. (27.8° C.) all year, is an obvious exception to the above. Perhaps the manini population in these islands has differentiated in this respect, although the Phoenix group is not markedly isolated.

In order to preclude the possibility that the different transformation sizes of the different areas are entirely due to genetic factors (reflecting possible races of the manini in all these areas) a comparison was made of the size at transformation of specimens obtained at Oahu in an early cool and a late warm part of the season. Thirty-nine first-day transforming manini were obtained in collections from April 11 to May 8, 1953. The standard lengths of these specimens are plotted as the solid line of Figure 16. The dotted line enclosing the stippled portion of the graph represents the lengths of 38 first-day transforming manini taken from July 7 to October 4, 1953. The average sea surface temperature at Oahu from February to May is 75.7° F. (24.2° C.); the average from July to October is 79.1° F. (26.2° C.), based on average temperatures from 1941 to 1947 presented by Leipper and Anderson (1950). The mean length of the April–May group is 26.256 mm.; the mean length of the July–October group is 25.447 mm. Although the difference in length appears highly significant, a t test was made by the group comparison method (Snedecor, 1948: 80). The resulting t value of 3.11 gives a probability of nearly 0.001 of obtaining a value greater than this; thus it is extremely unlikely that samples as diverse as the above could be

TABLE 7

SIZE OF *Acanthurus triostegus* AT TRANSFORMATION FROM THE POSTLARVAL TO THE JUVENILE STATE

LOCALITY	DATE	NO. SPECIMENS	STANDARD LENGTH (mm.)	
			Range	Mean
Durban, S. Africa	May 8	2	23–25	24
S. India	Jan. 19	1	22.5	22.5
East Indies				
Sumatra	Dec. 19–25	1	21	21
Moluccas	——	10	19–21	20.2
New South Wales	——	3	24–25	24.3
Palau Is.	Aug. 3–6	7	21–23	22.1
Philippine Is.	Jun.	1	23.5	23.5
Okinawa	Sep. 6–8	2	22	22
S. Japan (30.4° N.)	——	2	21–23	22
Ningpo, China (29.5° N.)	——	5	24–26	24.9
Mariana Is.				
Guam	Jun. 28	3	22–24	22.7
	Jul. 24	7	20.5–23	21.8
	Nov. 25	2	21.5–23	21.25
Saipan	Jul. 9–14	1	22	22
Rota	Nov. 11–13	16	21.5–24	22.5
Marshall Is.				
Bikini	Apr. 1	1	23.5	23.5
	Aug. 17–18	2	22–22.5	22.25
Eniwetok	May 20	2	23–23.5	23.25
Kwajalein	Jun. 24	7	22–24	22.7
	Sep. 1	3	22–24	22
Onotoa, Gilbert Is.	Aug. 21	9	21–25	23.2
Samoa Is.				
Tutuila	Jun. 3	1	22	22
Rose	Jun. 11–14	1	24	24
Swains	May 3–9	1	25	25
Phoenix Is.				
Canton	Apr. 15–28	3	25–26.5	25.8
	May 13	3	25–27	25.8
Enderbury	May 15–19	7	25–27	25.3
Hull	Jul. 7–17	9	23–25.5	24.1
Howland I.	Sep. 24	8	23.5–26	24.3
Line Is.				
Palmyra	Jan. 18	1	23.5	23.5
Fanning	Aug.	1	24	24
Wake I.	Jun. 9	2	23.5–24	23.75
Marcus I.	Aug.	4	25–26.5	25.6
Marquesas Is.	Feb. 3	1	26	26
	Aug.	2	26–26.5	26.25
Makatea, Tuamotus	Feb. 13	3	23.5–25	24.3
	Mar. 15	8	23–25.5	24.4
Mangareva	Feb. 3	7	24–25	24.7
Tahiti	Feb.	1	26.5	26.5
	Apr. 28	1	23	23
	Jun. 16	11	22.5–26	24.2
	Jul. 8	2	23.5–24	23.75
	Aug. 12	4	22.5–24	23.4
	Aug. 28	5	23–25.5	24.4
	Oct. 4	3	25–27	25.7
	Dec. 30	17	22.5–26.5	24.5
Clarion I., Mexico	——	1	25	25

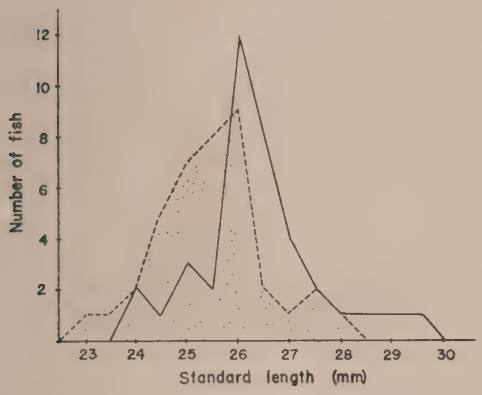

FIG. 16. Size of *Acanthurus triostegus sandvicensis* from Oahu during the first day of transformation from the acronurus to the juvenile stage. The solid line represents all of the specimens collected from Apr. 11 to May 8, 1953; the dotted line indicates those collected from Jul. 7 to Oct. 4, 1953.

drawn by chance from a population in which the transformation length was the same throughout the season. It is therefore concluded that a relationship as indicated above exists between the size of transformation of the manini and the temperature of the water.

Breder (1949*a*) stated that the complete transformation of *Acanthurus chirurgus* occurred in a period of about 48 hr. He did not, however, define the morphological state that constitutes complete transformation. In the manini, adult coloration is nearly acquired after about 48 hr., but other major changes are less than half completed after this length of time. Admittedly it is difficult to define a precise stage at which larval characteristics have completely disappeared and miniature adult structure is assumed. Probably the most convenient criterion is the stage at which the scales have completely formed. Drawings of developing scales have been made by Poey (1875: pl. 3) for the West Indian species, *Acronurus caeruleatus* (= *Acanthurus coeruleus*) and *Acronurus nigriculus* (= *Acanthurus bahianus*). The first evidence of the formation of the scales is the appearance of scattered thornlike outgrowths which project upward as well as posteriorly from the narrow, vertical, fleshy ridges of the body (these spines do not occur on the anterior half of the body). The spines are present on manini acronuri caught offshore at a night light, but they can be seen on these specimens only with the aid of a microscope. They enlarge and become visible (when wet) with the naked eye on the majority of first-day transforming individuals as early as 7:00 A.M. Each such spine on later stages was found to be a central stout ctenius of a single developing scale; the majority of scales, however, do not possess these. As scale development proceeds, the large ctenii flatten and become overlaid with epidermis. When this occurs, scale development is essentially complete as are other major changes of the transformation process. Scale development normally requires 4-5 days. In an aquarium the whole process seems delayed, and about 6 days are necessary.

Figure 17 shows transforming manini on the first, second, third, and fourth to fifth days. The second-day and third-day stages were identified with assurance by comparison with specimens recovered in tide pools following fin clipping on the first day of transformation. No older marked specimens were recovered at the stage when scale structure is just complete, and the time this required had to be inferred from the percentage of specimens in the different stages collected in the weekly poison stations of the Diamond Head tide pool of Figure 4.

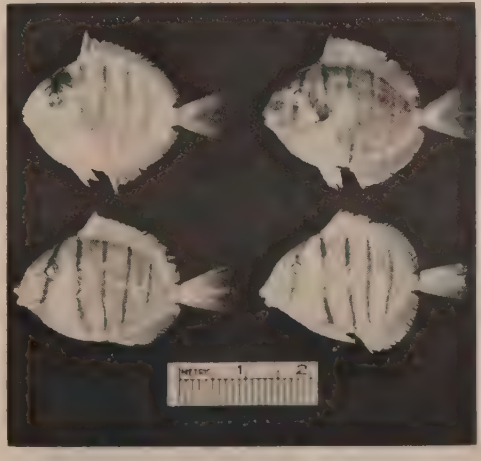

FIG. 17. Transformation of *Acanthurus triostegus sandvicensis* from the acronurus to the juvenile stage. Upper left, first day; upper right, second day; lower left, third day; lower right, fourth or fifth day.

The vertical dark bars which are so characteristic of the manini are absent from the majority of night-light specimens. They are faintly visible on a few such specimens, however. Manini caught in tide pools during the same night when they came in usually possess traces of the dark bars, but some, presumably very recent arrivals, totally lack these markings. One of the two previously mentioned specimens which were observed just entering shallow water from deeper areas of the Ala Wai Yacht Basin had traces of the bars, and the other lacked them. The bars are not dark on any of the specimens collected at night. During daylight of the first day of transformation the bars become intensely dark brown. This suggests that sunlight might accelerate or perhaps even be necessary for the darkening. In a review on the biochemistry of the production of melanin, Lerner and Fitzpatrick (1950: 119) pointed out that ultraviolet irradiation appears to be concerned with melanin formation in at least four different ways, all of which tend to increase pigmentation.

An experiment was designed to determine whether any changes occurred in the transformation process when light is excluded. Two transparent specimens which displayed no evidence of dark vertical bars on the body were taken at midnight. These were placed in a battery jar containing 2½ gal. of aerated fresh sea water. The jar was covered with a box which, in turn, was covered with a heavy black cloth. Three other transparent specimens taken at the same time were placed in a aquarium of about the same capacity which was not darkened. The battery jar was uncovered 12 hr. later. The transformation of the two specimens had barely started. The stage which was reached was about comparable to transforming fish taken shortly after dawn. The bars on the side were barely discernible and the ctenii of the developing scales on the posterior half of the body were just visible. One of the fish was on its side, dying. It was removed, and the jar was again darkened. Eight hours later the second specimen was found nearly dead. The bars were possibly a little darker; the transparency of the body was largely replaced by opaque white, an abnormal color alteration. The three aquarium specimens completed their transformation. The results suggest that light is essential to normal transformation. To be conclusive, however, the experiment should be repeated several times. If light were conclusively demonstrated to be essential to the metamorphosis of the manini, the mechanism by which it exerts its effect would still not be known. It might act directly on the tissues, or by way of the visual sense, or both.

During the first day the transparency of the body is gradually lost. By the end of the day the dark brown bars on one side of the body cannot be seen through the body from the other side as they may be on specimens such as the one in Figure 14. The bright silver color on the abdomen and head is slightly dulled by the end of the day due to the development of melanophores external to the silver layer. The iridocytes causing the silver sheen on the abdomen are present in the peritoneum. On the gill cover they occur in a layer on the underside of the opercular bones. They are found on the surface of the cleithrum at the edge of the gill opening. No silver color is present dorsal to the eye. Instead there is a broad black band which covers the brain. This is found beneath the dorsal bones of the head. By the third day both the silver color and the black band on the head are nearly imperceptible. They are, like the rest of the body except the region of the dark bars, white with numerous, close-set, small flecks of dark brown. The olivaceous cast of the body as seen on juvenile and adult manini is not conspicuous until about the fourth or fifth day of transformation, probably because of slow development of yellow chromatophores.

The second dorsal and second anal spines of the acronurus stage are longer and stouter than the other spines of these fins (Fig. 14). If stuck by either of these spines or by the pelvic spines, a stinging sensation almost comparable in intensity to a bee sting is felt. It is believed that these same spines, very elongate in the 6.6 mm. manini, are venomous in earlier stages. The poisonous nature of the spines appears to be a larval adaptation, for it is lost by the third day of transformation. A diminution in the relative length of the second dorsal and second anal spines may be seen in the series of transforming specimens of Figure 17. The relative change in length of these spines continues until, in late

juveniles and adults, the third dorsal and third anal spines are much longer than the second dorsal and second anal spines, respectively. The latter, however, remain thicker. All of the acronurus spines are T-shaped in cross-section; all are solid. The nature of the venom apparatus is unknown. The T-shape of the spines persists into the juvenile stage.

One of the most striking changes during transformation is the alteration of the configuration of the head. This consists primarily of an increase in the length of the snout, resulting in the mouth being in a more ventral position. The rostral prolongation may be seen in Figures 14 and 17. The diameter of the eye (which does not change during transformation) of the transparent specimen of Figure 14 is slightly longer than the length of the snout; in the just transformed manini the eye diameter is contained about 1.5 times in the length of the snout. The osteological changes which accompany the changes in the proportions of the head were not investigated.

A slight increase in standard length occurs during the metamorphosis to juvenile form. After 5 days in an aquarium, three transforming specimens, 24.1–25.0 mm. in standard length, increased 1.2–1.5 mm. in standard length. Most of the increase is attributable to the prolongation of the snout.

A remarkable lengthening of the digestive tract takes place during transformation (Fig. 3). In a period of 4 to 5 days the total length of the alimentary tract of the manini increases about three-fold. This lengthening occurs at the time of a change in food habits from feeding on zooplankton to feeding on algae (see pp. 222–223).

The teeth of the manini acronurus just before transformation are very different from the simple conical teeth of the postlarval manini (as seen in 6.6 to 7.0 mm. specimens). They are close-set and flattened with denticulations on the edges similar to adult teeth. There are about 14 of these teeth, each with 6 or 7 denticulations, in the upper jaw with a broad gap between teeth at the symphysis of the jaw. There are 14 such teeth in the lower jaw with 5 denticulations each. During transformation 8 large adult-type teeth (with about 12 denticulations), which are preformed within the premaxillary bone above the upper series of larval teeth, grow down over the larval teeth which are shed. The more medial of these large teeth are the largest and the first to emerge. In the lower jaw the same process occurs. Six large adult-type teeth with 7 denticulations grow over the larval teeth. Larval teeth lateral to these large ones may persist beyond the period of transformation.

The sequence of tooth replacement is not without variation from specimen to specimen. At the stage when the scales are just completely formed on the body some specimens have fewer than 8 upper and 6 lower teeth in position. Some specimens taken at night lights in deep water appear to be getting their adult teeth prematurely. One specimen of *Acanthurus triostegus triostegus* obtained at a night-light trap at anchorage at Christmas Island already had 6 large upper and 2 large lower teeth.

GROWTH

No information could be found in the literature on growth of any species of surgeonfish. The closest family, phylogenetically, to the Acanthuridae in which growth data are available for comparative purposes is the Teuthididae (Siganidae of most authors). Like the acanthurids, the teuthidids are herbivorous (Suyehiro, 1942; Al-Hussaini, 1947). Whitehouse (1923: 68–70, 83–85) reported that juveniles of *Teuthis java* grew from a length of 25 mm. in April to 75 mm. in September in Silavatturai Lagoon, Tuticorin, India. Ommanney (1949: 48–49) stated that *Siganus corallinus* (= *Teuthis corallinus*) reached a length of 180–220 mm. after 2 years' growth, and about 280 mm. at the end of the third year, in Mauritius.

Information on growth of the manini was obtained by the rearing of fish in a tank of the Hawaii Marine Laboratory, at Coconut Island, Oahu, analysis of the progression of modes among the juveniles of Figure 6, recovery of young fish marked by fin clipping, and the recovery of tagged adults. The data are discussed separately under these headings below.

The standard-length measurement for all of the work on growth of the manini is the length from the tip of the snout to the middle of the

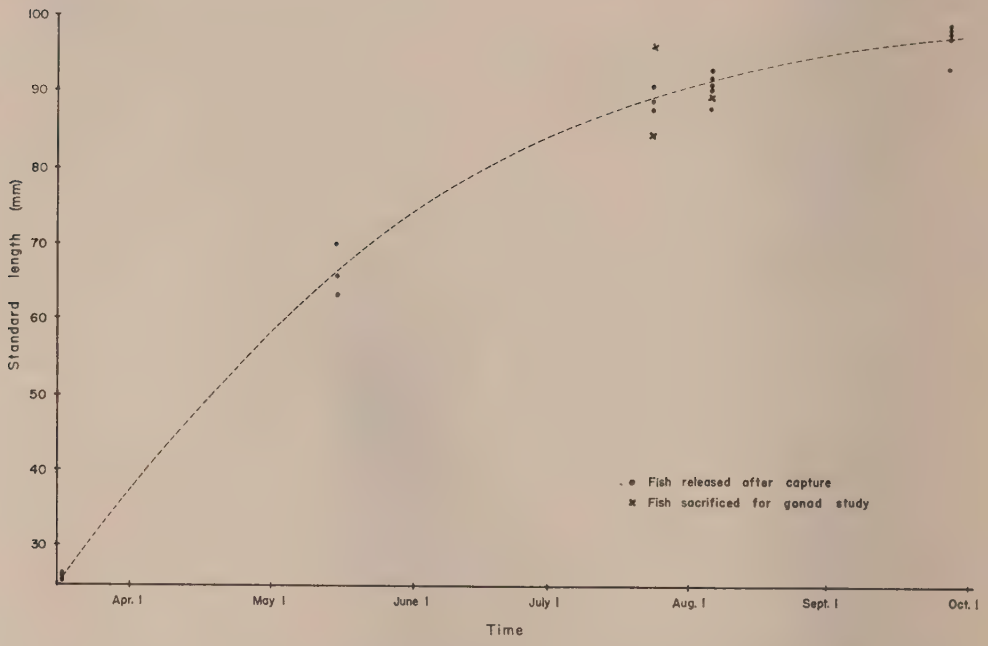

FIG. 18. Growth of *Acanthurus triostegus sandvicensis*. Based on 10 juvenile specimens, 25 to 27 mm. in length, released in a concrete tank at Coconut Island, Oahu, Mar. 17, 1953.

dorsal black bar posteriorly on the caudal peduncle. It was found that this measurement could be made with greater accuracy on living fish than the usual standard length to the base of the caudal fin (i.e., posterior edge of hypural plate). The original measurements are herein retained for all tables, graphs, and discussion (and designated simply "standard length"), rather than converting to true standard length. Should the latter measurement be desired, it may be obtained by increasing the peduncular bar length by 3.5 per cent.

Growth of Captive Fish

Ten manini, 25–27 mm. in standard length (mean length 26.4 mm.), were placed in a concrete tank at Coconut Island on March 17, 1953. The tank measures 34.7 × 10.8 ft. and is an average 3.9 ft. in depth. A constant supply of sea water is pumped into the tank. When the fish were introduced, no other fishes were present and the walls were covered with an exceedingly luxuriant growth of many species of algae. The top of the caudal fin of the 10 juveniles was removed to permanently mark them (see below) as a safeguard against the possible addition of more manini to the tank by other persons.

The growth of these fishes is recorded in Figure 18. The 3 fish captured on May 13 (mean length 66.0 mm.) had grown an average of 20 mm. per month since March 17. On August 6 all of the manini were caught (2 had disappeared and 3 were killed for gonad study). They averaged 90.9 mm. in standard length. By September 27 they had grown to an average standard length of 94.3 mm.; thus the average rate of growth from August 6 to September 27 had diminished to 1.8 mm. per month.

The manini were caught by seining the tank after partially draining it. The efforts to catch them on September 27 failed several times, and much of the algae was detached from the walls of the tank before all of the fish were netted.

Following this the amount of algae on the walls remained slight and apparently insufficient to support the growth of the fish. The 5 remaining manini were measured again on November 19. They were thin, and instead of growing they lost an average of 1.3 mm. in standard length. Manini this size shrink nearly 3 mm. in standard length when preserved in 10 per cent formaldehyde solution.

Growth Measurement from Progression of Modes

The collections of juvenile manini from Kewalo Basin to Makapuu Point, Oahu, plotted in histogram form in Figure 6 permit an estimate of the early growth rate by analysis of the progression in standard length of prominent modal groups from week to week. By this method the growth rate of juvenile manini from about 26 to 45 mm. in length is computed at about 12 mm. per month. Particular reference was made to the mode which first fully appears on the graph on April 17.

Growth of Marked Juveniles

Before undertaking the marking of juvenile manini in the field by fin clipping, experiments were performed in aquaria to test the possibility of fin regeneration. One pelvic fin and the posterior part of the soft portion of the dorsal fin were cut from 3 juvenile manini averaging 27.5 mm. in standard length. Although the fins were severed at the juncture with the body they regenerated rapidly and were nearly completely formed in a month. In a second experiment the top few rays of the caudal fin were cut away as well as the upper corner of the hypural plate to insure the removal of all fin elements. This cutting resulted in the permanent deformation of the caudal fin of the fish.

Ten manini, 28.5 to 30 mm. in standard length (mean 29.25) were caught at the Ala Wai Canal near its entrance to the Ala Wai Yacht Basin on April 24, 1953. The top of the caudal fin of these fish was removed and they were released in the same area. Three of these marked fish were recovered, 1 on May 2 which measured 32.5 mm. in standard length and 2 on May 15 which were 34.8 and 37.0 mm. long (Fig. 19). The average growth rate was 9.6 mm. per month.

Ninety-six juvenile manini, 25–28 mm. in standard length (mean 26.6 mm.), were caught, marked, and released in a Diamond Head tide pool on May 10, 1953. A total of 11 were recovered from this pool on three different dates (Fig. 19). The average growth rate of these fish was 12.9 mm. per month. The 4 manini of this group which were recovered on June 23 ranged from 42.5 mm. to 48 mm. in standard length and averaged 45.1 mm. When first caught with the 92 others on May 10, these fish were a part of the prominent mode which may be seen centered on about 26.5 mm. standard length on the graph for this date in Figure 6. This same mode is still apparent in a sample of 147 fish which were collected at Diamond Head on June 23 in an effort to recover marked fish. At this time the mode is composed of 27 fish, 40 to 49 mm. in standard length with a mean of 43.3. Since the 4 marked manini taken on this date are distributed well within the mode and of larger average size than its mean value, it is concluded that the fin clipping did not retard the growth.

The difference in growth rate between the Diamond Head marked manini and the Ala Wai marked fish may be associated with the difference in algal flora of the two areas. Algal growth in the latter area is sparse; it consists primarily of diatoms. The extraordinary growth rate of the captive manini in the Coconut Island tank is probably also associated with the nature of the algal food supply; the stand of algae was much greater than normally observed in reef or bay areas around Oahu. Invertebrates, including herbivorous types such as echinoids, gastropods, and decapod crustaceans, were present in the tank. It is assumed that the large supply of algae was primarily due to the lack of herbivorous fishes. When introduced to this unusual environment, the 10 juvenile manini were given an unprecedented opportunity for rapid growth.

In view of the more normal environment of the Diamond Head tide-pool region and the similarity in growth rate of marked fish from this region to the rate computed from the progression of modes, 12–13 mm. per month is

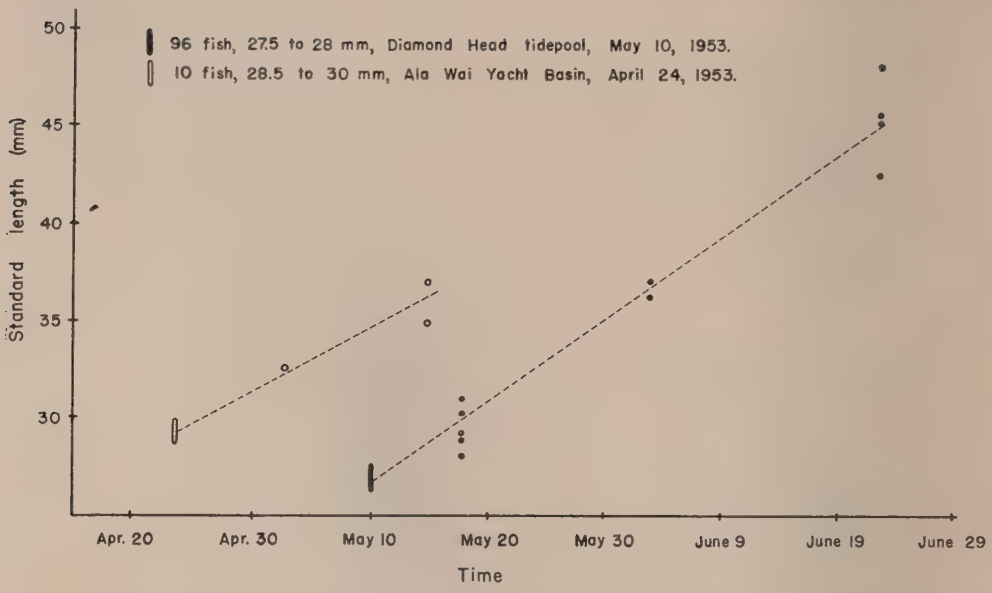

FIG. 19. Growth of juvenile *Acanthurus triostegus sandvicensis* on Oahu. Based on recovery of marked fish.

considered as the probable rate of growth in spring of early juveniles of the bulk of the Oahu manini population.

The growth of juveniles in the winter ceased. This was first noticed from shallow-water collections and observations made during the fall and winter months of 1952–53. Up to November, manini less than 40 mm. could usually be found in the collections or seen in the water. In late November, December, and January no specimens less than 40 mm. were found, but the 40 mm. size persisted. This suggests that the last incoming juveniles of the season, which enter tide pools in early October, grow to a standard length of about 40 mm. by November and then stop growing. During the next two winters the same phenomenon was noted.

In order to demonstrate this cessation of growth and to ascertain the time when growth is resumed, 22 juvenile manini, 40 to 72 mm. long, were caught with a dip net, marked by fin clipping, and released on the nights of January 5 and 6, 1954, at the entrance of the Ala Wai Canal to the Ala Wai Yacht Basin, Oahu. Fish were taken at both the northwest (Waikiki Yacht Club) and southeast shores of the canal entrance. On the two sides of the canal three basic methods of fin clipping were utilized: the top of the caudal fin was removed, the bottom of the fin, and both the top and bottom. Manini of variable size were chosen for each category, and another fin was cut on each specimen. Also any distinctive color marks (usually at the base of the pectoral fin) were noted.

Subsequent efforts to recover the fish were limited by conditions of wind, tide, and turbidity of the water. Although more subject to the effects of turbidity than exposed shores, the Ala Wai area was preferred, for fish are more restricted to the shore area because of the mud bottom away from the bank of the canal. Fish of this size in a region such as Diamond Head move well out on the reef where the broader area, deeper water, and action of surf make them difficult to capture and recapture. Although, as mentioned, some of the larger manini come into tide pools at night, their numbers are not sufficient for such a marking program.

Since fish were measured alive with a light at night, difficulty was experienced in obtaining

precise length determinations. Although the lengths were recorded to 0.5 mm., it should be added that variations as great as 1 mm. from the original measurement were noted in unbiased tests of accuracy.

Eleven of the 22 marked fish were recovered; all from the area of release. Two of these were recaptured a second time. Of the 9 fish taken before February 1, few, if any, showed significant growth (Table 8). The 4 recovered on February 21 and 27 displayed a definite increase in size, indicating a resumption of growth during this month in the area tested and for the year 1954.

One other marked fish caught in February and 2 in early March had regenerated the fin other than the caudal fin which had been cut and could not be distinguished from at least 1 other fish of their respective caudal fin categories.

Although there is some indication of a relationship between increasing water temperature and the onset of growth of these manini, the temperature data are inadequate to establish any definite correlation. Temperatures were taken in the canal at night between 9:30 and 12:00 P.M. about 3 in. beneath the surface (Table 9).

It should be emphazied that the temperature at the mouth of the Ala Wai Canal probably varies much more in a day or from day to day than the sea surface temperature off Oahu (mean water temperatures for Honolulu in 1955 are given in Hydrographic Office, 280, as follows: January 24.0; February 23.6; March 24.1). The Ala Wai area is not large and the water is therefore more subject to change due to variation in insolation and atmospheric temperature than the open sea. Probably more important are the changes produced due to variation in the run off of water from Palolo and Manoa valleys which drains to the canal and to the ever variable tides which affect this estuarine region. In order to provide adequate data for the determination of a correlation, temperatures should have been taken at regular intervals each day and an analysis of growth made in terms of average daily water temperatures.

TABLE 9

TEMPERATURE AT THE MOUTH OF THE ALA WAI CANAL

DATE	TEMP. (° C.)
Jan. 5	22.8
Jan. 20	23.2
Feb. 1	23.1
Feb. 27	25.3
Mar. 2	24.7
Mar. 21	24.9

Growth of Tagged Adults

The first tag used on the manini was the Atkins type (Rounsefell and Kask, 1945: 330, fig. 1). It consisted of a single rectangular piece of white cellulose acetate, 5 × 28 mm. in size, and was attached through the back of the fish beneath the posterior part of the dorsal fin with 4-lb. test monofilament nylon. Two small adult

TABLE 8

RECOVERY SIZE OF JUVENILES OF *Acanthurus triostegus sandvicensis* MARKED ON JANUARY 5–6, 1954

STANDARD LENGTH WHEN MARKED (mm.)	STANDARD LENGTH WHEN RECAPTURED				
	Jan. 20	Jan. 26	Feb. 1	Feb. 21	Feb. 27
40.0	41.0				
46.5	47.0				
49.0					54.5
51.5				57.0	
55.0	55.5			60.0	
55.5	55.0				
56.0		56.5			
64.0			64.0		
66.5		66.0			
70.5	70.5				
72.0	72.0			79.0	

manini were tagged and held in a tank at Coconut Island. Within 3 weeks the tags were no longer present on the fish. They were recovered from the bottom of the tank with the loops of nylon intact, thus indicating that the nylon had pulled through the flesh.

The same type of tag was then threaded through the upper part of the hypural plate of the 2 fish. As they swam, the tag of each oscillated slightly as it was drawn along in a position just above the caudal fin. Three months later both tags were in place, and the method looked auspicious. On March 3, 1953, while operating from the fishing vessel "Friendly Isle" owned by Samuel Kaolulo, 50 adult manini were tagged in this manner. Two fish were recovered 5 days later, both in the vicinity of the point of release. No other recoveries were reported. The two Coconut Island manini lost their tags before 6 months elapsed, and further plans for tagging by this method were abandoned. Disappointing results with Atkins tags have been reported by other authors, such as Fraser (1955).

Petersen disk tags were then tried. Since stainless steel seemed most promising among the metals commonly used to attach these tags (Calhoun, Fry, and Hughes, 1951), this material was chosen for initial trials. Considerable difficulty was experienced, however, in bending the free ends of the wire on a struggling fish, a disadvantage clearly pointed out by Calhoun *et al.* Attention was then shifted to nonmetallic substances, of which nylon seemed the most propitious. It is superior to metal in its pliability, nontoxicity, and in not being subject to electrolytic action or fatigue. In order to keep the disks in place, the ends were melted into knobs with a flame (Fig. 20). The position of the tag on the fish is shown in Figure 21. (For a detailed discussion of this tagging method, see Randall, 1956*a*.)

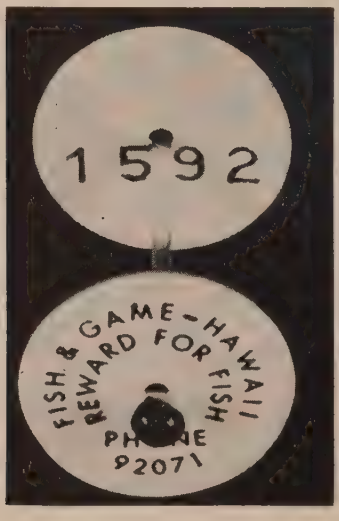

FIG. 20. Petersen disk tag used in tagging *Acanthurus triostegus sandvicensis* on Oahu. The ends of the monofilament nylon connecting the disks have been melted into knobs with a cigarette lighter. The heating caused a blackening of the one knob which is visible.

On August 6, 1953, 3 captive adult manini were tagged with plastic disks by 60-lb. test monofilament nylon (diameter, 1 mm.) and 1 with stainless steel wire.

Seven weeks after tagging, all 4 fish, along with 6 untagged fish which were measured at the time the 4 fish were tagged, were caught and remeasured. The 6 untagged fish, which had a mean standard length of 86.3 mm., had grown an average of 6.6 mm. The 3 tagged with nylon (mean standard length, 84.4 mm.) grew an average of 7.7 mm. The 93 mm. fish whose disks were attached with stainless steel wire added the smallest increment of growth, 2.5 mm. Although there are insufficient data for definite conclusions, it seems apparent that manini tagged with Petersen disk tags fastened with monofilament nylon are capable of normal growth.

One of the fish which survived 11 months until July when the tank was needed for other purposes still retained its disks fastened with nylon. It was noted that the tagging wound was small, and there was no evidence of deterioration of the nylon.

Since the experimental tagging demonstrated no obvious fault in this method, field tagging was begun. Forty-five manini were tagged with hand-made tags. This proved to be most time consuming, and assistance was requested of and granted by the Hawaii Division of Fish and Game. Manufactured tags of laminated cellulose acetate (Fig. 20) were purchased. In order to publicize the tagging, posters (Fig. 21) were

REWARD !
IF A MANINI IS CAUGHT WHICH IS TAGGED AS SHOWN IN THE ABOVE DRAWING, PLEASE CONTACT FISH & GAME WARDEN OR BRING THE FISH TO THE DIVISION OF FISH & GAME (PHONE 92071). ONE DOLLAR ($1.00) REWARD WILL BE GIVEN.

FIG. 21. Poster used to publicize tagging of *Acanthurus triostegus sandvicensis* on Oahu.

distributed by game wardens. Carl M. Nemoto, then working for the Division of Fish and Game, tagged a total of 1,157 manini, 70 to 152 mm. in standard length, from July 6 to November 23, 1954. Forty-five additional fish were tagged on May 4 and June 6, 1955. The tagging was carried out from Kaolulo's vessel between Kewalo Basin and Koko Head, Oahu. The manini were caught in traps, tagged, measured, and released at the place of capture, which was recorded.

Due to the difficulty of using a flame when tagging from a vessel exposed to the strong winds of the Kaiwi Channel (Molokai Channel), Nemoto decided to tag most of the fish by crimping brass leader sleeve on one end of the nylon (the other end was melted into a knob prior to the departure of the vessel). This method was devised by Kenji Ego of the Division of Fish and Game.

Of the total of 1,247 manini tagged with disk tags, there have been 281 recoveries. Of the recovered fish, 173 have been returned alive, providing the opportunity for re-release. Twenty-four of these have been caught again, and 3 have been taken a third time (these recoveries are included in the 281 mentioned above).

The following table shows the lapse of time by 2-week periods between the release and recovery of tagged manini.

The fish with the longest recovery time was tagged on September 21, 1954, and recovered on July 29, 1955.

The explanation of the large number of manini recovered in the first few weeks following release and the sharp drop in returns thereafter probably involves a number of factors, such as mortality, loss of tags, and movement from the immediate area of release.

It is known that at least some loss of tags has occurred, for manini with the tagging wound but without tags have been noticed by fishermen. Since no fish have been seen with a tag in the process of moving up through the back and dorsal fin, it is assumed that most of the early losses, at least, occurred from a dropping off of a disk because of the knob being too small or the brass inadequately crimped. Analysis of the returned tags indicated that the knobs were too small in many cases. When the diameter of the hole in the disk is 1 mm., the knob should measure at least 2 mm.

TABLE 10

RECOVERY TIME OF TAGGED ADULTS OF *Acanthurus triostegus sandvicensis*

WEEKS FOLLOWING RELEASE OF FISH	NO. OF FISH RECOVERED
2	96
4	57
6	42
8	25
10	17
12	9
14	6
16	5
18	4
20	4
22	3
24	5
26	2
28	2
30	2
34	1
40	1

Movement from the trap area could account for a diminution in recoveries of tagged fish; however, evidence is strong against at least extensive migration by this species (see section on migration).

The marked drop in number of recoveries still seems too high to be accounted by any or all of the above reasons. One other possibility exists which is supported by the extraordinary high percentage of early returns. A tagged manini may show a greater predilection to re-enter a trap than untagged manini to enter a first time. This phenomenon has been conclusively demonstrated by Lawrence (1952) for bluegill sunfish in Iowa farm ponds. If such a tendency exists, it might be expected to be greatest in the fish soon after release and gradually abate with time. One manini was caught, tagged, released near the next trap, and caught again in this trap a few minutes later.

The 173 tagged fish which were returned alive were measured alive a second time by Nemoto and released. The remaining tagged manini were measured after they had died, and usually not when fresh but following partial drying and/or freezing. Seventeen of the latter group were recovered within the first 2 weeks following tagging. These fish were all from 1 to 7 mm. shorter than when first measured. The average shrinkage was 3.53 mm. The average standard length of these specimens measured to the peduncular mark is 109.8 mm. The average shrinkage per millimeter of length is therefore 0.032 mm. A correction was made for each fish which was measured dead (unless freshly dead) by multiplying its recovery length by this factor and adding the product to the length.

A total of 70 tagged manini were measured alive a second time following recovery within 2 weeks of tagging. Surprisingly, 46 of these were from 1 to 5 mm. shorter than when first measured; 10 were the same size; 14 were from 1 to 3 mm. larger. The average "growth" was —1.06 mm. A t test applied to these data to ascertain the probability of obtaining such a distribution from a population in which no actual change in length occurred resulted in a t value of 5.1. With 69 degrees of freedom this gives a probability of less than 0.001 of getting a higher t value; thus the null hypothesis of no significant difference is rejected. Since the same person measured the fish both times, the reason for the significantly shorter length is not apt to be any bias in measurement. The shorter length is probably due to shrinkage from starvation in the traps. Manini which are caught in traps may have been held in the traps for a week or more. Although both measurements are made on fish caught in traps, the effects of confinement would be expected to be greater on fish spending another period in traps within 2 weeks of their first.

In order to test the hypothesis that actual shrinkage occurs during starvation, 17 trap-caught adult manini (none showed evidence of long sojourn in traps), 112 to 131 mm. in standard length, were tagged, measured, placed in two large aquaria, and allowed to starve. Eleven days later the 5 fish which survived were remeasured. A definite shrinkage of from 1 to 5 mm. (mean 3.4 mm.) had taken place. The measurements were made by Nemoto.

The large amount of variation (—5 to +3 mm.) in the series of differences between the measurements made on the 70 fish when tagged and recovered within 2 weeks was unexpected. When it is realized that five different sources contribute to this variability, it is more comprehensible. There is the possible error in the first measurement of a living fish. The second measurement of this same fish constitutes an independent source of error. The shrinkage due to starvation is a third. Some manini which are caught have not been in a trap more than a few hours and will not have shortened at all; others held prisoner for a week or more may have shrunk several millimeters. Some manini initially caught after a very short stay in a trap, tagged with a minimum of injury, and recovered a full 2 weeks later after another brief period in a trap might have grown about a millimeter; thus growth itself probably forms a fourth source. An important fifth source of error in the measurement of the dead tagged fish is the variation in shrinkage after death. All of this variability has necessitated a statistical approach to the analysis of the data, and for this purpose the large number of recoveries has been most welcome.

Only those manini recovered after spending

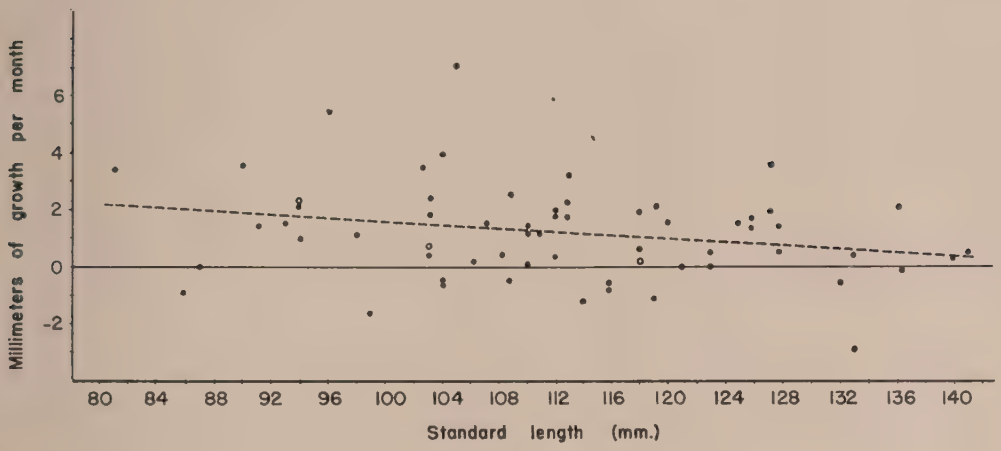

FIG. 22. Growth of *Acanthurus triostegus sandvicensis* on Oahu during the period Jul. 6 to Nov. 19, 1954. The points represent growth increment of tagged fish recovered after at least 1 month in the water. Circles indicate the superposition of one point on another. The dotted line is the regression of growth on standard length.

a month or more in the water following being tagged were utilized for growth study. In Figure 22 the data from 68 such fish, tagged between July 6, 1954, and September 28, 1954, are plotted. The dotted line represents the regression of millimeters of growth per month on standard length. The regression coefficient "b" (slope of the regression line) is —0.0292. The *t* value testing whether this regression differs significantly from zero is 2.085. With 66 degrees of freedom, the probability of getting a larger value of *t* is 0.04; thus the null hypothesis of no significant regression is rejected.

The comparison of the regression line with the upper part of the growth curve of Figure 18 is favorable. The average growth rate of the captive fish from August 6 to September 27 was given as 1.8 mm. per month. The average rate for this size range (90.0 to 94.3 mm.) from the regression line is 1.74 mm. per month.

In order to further confirm the finding of cessation of growth in winter, 155 manini were tagged on November 19 and November 23, 1954, by Nemoto. Twenty-five of these fish were recovered from December 23, 1954, to February 18, 1955 (see Fig. 23). Their average rate of growth is —0.112 mm. It therefore seems likely that no growth occurred during this period.

Tagged manini, recovered after a month or more in the sea, which were at liberty during winter and either fall or spring months have not yet been considered. Data from these fish are presented in Table 11.

If it is assumed that these 22 fish did not grow during any part or all of the 3-month period beginning November 19, and the growth rate of each is computed only from remaining months, an average growth rate of 1.8 mm. per month is obtained. This compares favorably with the rate of growth as indicated in Figure 22 (the average standard length in Table 11 is 110.6 mm.).

AGE

In view of the lack of winter growth of the manini it was hoped that annuli might be detected on the scales; however, none could be seen. Even the circuli of the tiny scales of this species are not very distinct. Cross-sections of vertebrae and otoliths were also examined, but with negative results. It was necessary to grind otoliths to about 1 mm. thickness and view them with subdued light under a compound microscope before any concentric rings could be seen. These were numerous and variable, and none were more sharply defined or grouped closer together than others.

TABLE 11

GROWTH OF *Acanthurus triostegus sandvicensis* RECOVERED AFTER 1 MONTH OR MORE FOLLOWING TAGGING

(Period of tag retention includes both winter and fall or spring months, 1954–55)

DATE RELEASED	DATE OF RECOVERY	STANDARD LENGTH	MEASURED DEAD	GROWTH (mm.)
Jul. 6	Dec. 14	105	×	3.4
23	Mar. 25	110		3.5
30	Dec. 23	115.5		5.5
Aug. 10	Feb. 6	113	×	7.8
10	Feb. 22	110		3.0
13	Jan. 28	98	×	3.1
27	May 17	97		8.0
Sep. 3	Feb. 6	101	×	7.2
3	Mar. 25	102		3.0
21	Jan. 24	98.5		4.0
21	Feb. 18	123	×	7.0
21	Feb. 21	105	×	7.4
21	Jul. 29	101		4.0
28	Dec. 23	121	×	1.8
28	Jan. 21	114		2.0
28	Mar. 25	122	×	1.8
28	Apr. 23	124		1.0
Nov. 19	Mar. 1	105	×	3.8
19	Apr. 5	152		1.5
23	Mar. 19	119		0.5
23	Apr. 27	105		3.0
23	Jul. 6	106.5		7.5

The next approach to obtaining the age of manini was the preparation of the length frequency curve of Figure 24. As the recruitment of the manini is seasonal, it seemed possible that year classes might stand out as modes on such a graph. At least for this curve, however, no definite modes are apparent. Probably the long period of recruitment (February to October), coupled with individual variation in growth, precludes the formation of distinct modal groups in standard length.

Age determinations of the manini would therefore seem possible only by rearing of fish, recovery of tagged fish (tagged at a size small enough to predict the age from growth rate with reasonable accuracy), or gross estimates from growth rate and knowledge of the maximum size.

Although the age attained by the manini is unknown, the following information on the age of two surgeonfishes supplied by Spencer Tinker, director of the Waikiki Aquarium, indicates that at least some acanthurids are long-lived. Two unicorn fish (*Naso unicornis*) which were present in the aquarium when an employee arrived in 1935, lived until they succumbed as the result of an accident in 1955. In 1940 several specimens of *Acanthurus xanthopterus* approximately 5–6 in. in length were placed in the aquarium. Two of these survived until 1951 in a small tank together; they had reached a size of about 20 in. At this time one slashed the

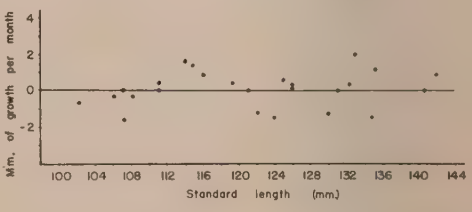

FIG. 23. Growth of *Acanthurus triostegus sandvicensis* on Oahu during the period Nov. 19, 1954, to Feb. 18, 1955, as based on the recovery of tagged fish which were in the water 1 month or more. The horizontal line was added at the zero point to facilitate comparison of the distribution of points with Figure 22.

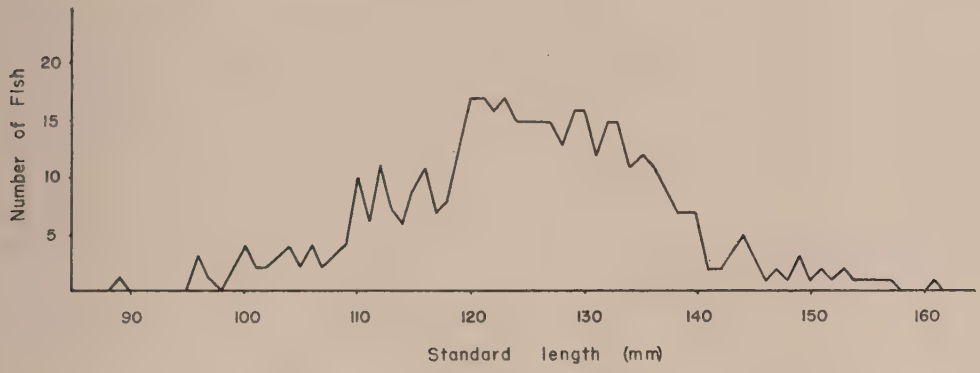

FIG. 24. Length frequency distribution of *Acanthurus triostegus sandvicensis*. Based on 426 specimens caught in traps from Kewalo Basin to Koko Head, Oahu, from Apr. 2 to 9, 1954.

other with its caudal spine and the latter ultimately died. The remaining fish died in 1955 because of an accident.

BEHAVIOR

Migration

Small juvenile manini tend to remain in the tide pool or shallow water area to which they first come from the pelagic state. Some evidence for this has already been presented. Of 176 juveniles taken in the weekly collections during a season from the tide pool of Figure 4, only 19 were 30 mm. or more in standard length and hence definite migrants to the pool from other areas. Since all of the fish in this pool were killed with rotenone each week, a much greater movement to such a large unoccupied area would be expected from the smaller adjacent pools if the species did not exhibit a tendency to remain in one location. When the adjacent pools were sampled for manini, they invariably contained many more specimens 30 mm. or larger, than less than 30 mm. It should be emphasized that these pools are isolated from one another for only about an hour or less during the infrequent low tides less than 0.0 ft. Even in areas like the shore of the Ala Wai Canal and the Ala Wai Yacht Basin where adjacent areas are freely available at all times and essentially identical in cover and food supply, appreciable movement does not take place. This is attested by the success in recovery of marked fish at the mouth of the Ala Wai Canal (Fig. 19, Table 8). Failure to take more of these fish was not due to their movement out of the area but to the difficulty in catching them. Less than one-fourth of the manini sighted were caught, and probably many others were hidden from view under large rocks, etc. None of the fish were taken farther than 20 ft. from the point of release, although the shore was searched as much as 100 ft. away.

On March 7, 1953, a juvenile manini, estimated 36 mm. in standard length, was seen next to the sea wall of the Ala Wai Yacht Basin beside a pier. It was probably among the first manini of the season to come into the area, and was the only individual this size visible for over 100 ft. of sea wall on either side of the pier. On the 9 different days up to March 28 when attempts were made to find this fish, it was seen. Its greatest movement from the place where first observed was only 12 ft. On March 28 it was caught (measured as 41 mm.), and the posterior part of the soft portion of the dorsal fin removed in order to identify it with greater certainty in further observations. It was found on 10 different days up to May 2, but never more than 12 ft. to either side of the pier. On April 24 the dorsal fin had almost completely regenerated and by May 2 the fin appeared normal.

As juvenile manini grow, they migrate seaward from the tide-pool zone (in the harbor area, as mentioned, the larger fish remain in shallow water because of the lack of cover and

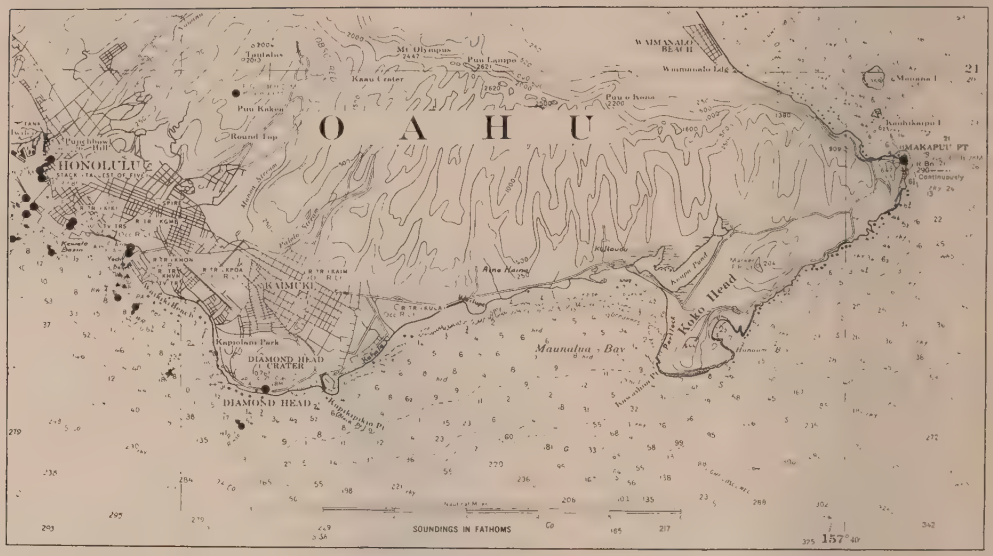

FIG. 25. Southeastern coast of Oahu, Hawaiian Islands (after U. S. Coast and Geodetic Survey Chart No. 4110).

the lack of algae on the mud bottom away from shore). This movement is demonstrated in the graphs of Figure 6. The relative size of any one modal group is progressively smaller in subsequent collections (predation would have to be extremely heavy to achieve the same diminution). The one seeming exception is the large collection of May 29. This, however, was made from a very large pool at Diamond Head which averages about 4 ft. in depth. A higher percentage of larger manini were expected from this collection than previous collections which were all made in pools less than 2 ft. deep.

Information on movements of adult manini was obtained from the recovery of tagged fish. Unfortunately, exact locality data for the areas of release and recovery were usually not available. Since Nemoto was operating from a commercial vessel solely by virtue of the kindness of Kaolulo, he could not delay the fishing to take bearings or soundings. Most locality data from fishermen who recovered tagged manini were also very nonspecific. As a result, little is known of local movements of adults, and only migrations from one major area to the next or the lack of such migrations could be ascertained.

Kaolulo's traps are arranged in groups, most of which are well separated. Nemoto designated these areas as offshore from the following: Yacht Basin, Royal Hawaiian Hotel (Waikiki), Natatorium (near Kapiolani Park), Diamond Head, Black Point, Kahala, KULA antenna, Wailupe, Aina Haina, Kuliouou, Niu Peninsula, Portlock, and Koko Head (Fig. 25).

Of the 281 recoveries of tagged manini 237 were made by Kaolulo; 126 tagged fish, most of which were early recoveries by Kaolulo, were returned to the Division of Fish and Game with no record of where they were caught. This difficulty arose because of his resorting to memory alone to recall the area where tagged fish were taken. When he caught several fish he usually admitted that he could not be sure where any of them were caught. He was finally persuaded to keep record of locality by tag number.

One hundred and thirty-two tagged manini were recovered which exhibited no definite migration. Ignoring fish released in Kewalo Basin for the moment, this leaves only 11 records which indicate that some migration might have occurred. Five of these were fish which were displaced from their home area by movement of

the vessel to the next area and which apparently returned to the home area. One was caught at Wailupe, released at Black Point, and caught again at Wailupe. The same fish was not released the second time until the Kuliouou area was entered. It was caught a third time back at Wailupe. Another Wailupe manini was released opposite the KULA antenna and was recovered at Wailupe. Two fish were caught at Black Point, released farther offshore at Kahala, and caught again at Black Point. The remaining 6 records are as follows: 1 fish supposedly migrated from Kuliouou to Wailupe; another went from the KULA antenna to Niu Valley; 2 apparently went from Kuliouou to Wailupe; the last 2 swam from Black Point to Kuliouou. The possibility of error must be kept foremost in mind for all of these apparent migrations. The 2 manini which moved from Black Point to Kuliouou were both caught by Kaolulo on the same day, which seems unusual.

In view of the manner in which the locality data have been reported, it is surprising that more alleged migrations have not taken place. It is therefore concluded that extensive migrations of this surgeonfish probably do not normally occur.

Of the 172 tagged manini which were released a second time 125 were set free on Kewalo Basin. Twelve of these have been recovered. One was taken between piers 7 and 8 in Honolulu Harbor. The rest were all captured either at Kewalo or in the Diamond Head direction. One was taken from a tuna boat in the basin, and 2 off the Kewalo breakwater. One locality reads Ala Moana and another Ala Wai. One manini was caught by Kaolulo with no record of locality. Since his nearest trap is off the Yacht Basin, this fish must have migrated at least this far. One manini released at Kewalo was caught at a depth of 30 ft. directly off the Royal Hawaiian Hotel. A fish from Black Point was caught 6 months later at wading depth off Diamond Head not far from Black Point. The most interesting recoveries of Kewalo-released fish are 2 from Kuliouou which were originally captured at Kuliouou and 1 at Wailupe which was first caught off Wailupe. Although, again, the possibility of error must be kept in mind, it seems unlikely that the only 3 examples of extensive migration (about 8 mi.), all of which occurred among the 12 fish recovered following release at Kewalo Basin, could be mistakes. One of the Kuliouou manini was recorded from this area by tag number by Kaolulo. When questioned of any chance of error, he insisted there could be no mistake. An interval of 3½ months elapsed between its release in Kewalo and its final capture. The other Kuliouou fish was obtained from the Young Market in Honolulu. The interval between the release and capture of this fish was 16 days. The Wailupe fish was recovered by Kaolulo 1 month after release in Kewalo Basin. The locality was reported verbally. The intriguing interpretation of these 3 records of long migration is that at least some manini removed from their home area will continue to wander on the reef until they return. Three records are too few on which to base such a conclusion; therefore more data should be obtained by purposely displacing tagged manini considerable distances from the original area of capture.

Aggregations

Adult manini are often observed in large feeding aggregations which slowly move over the bottom. These groups of fish may be dense and cover areas more than 50 ft. in diameter. What appears to be the same school was repeatedly seen in the same general location at the northwestern end of Manana Island. This group of fish was observed in the area in fall as well as during the breeding season. The behavior of individual manini of the school did not seem to differ from that of solitary adults.

One school of about 300 adult *Acanthurus triostegus triostegus* was observed in the northeast side of the pass at Takaroa, Tuamotu Archipelago, over a period of a week. The school moved as a close-knit mass several feet off the bottom, stopping frequently for periods of several minutes to graze algae. One or two *Aulostomus chinensis* were observed to accompany the school, and the manini were almost constantly harassed by *Acanthurus glaucopareius* which chased individual fish. The school moved as much as 300 yd. in several hours.

Juvenile manini are gregarious and are often seen swimming in groups. One group of 11 individuals, all less than about 36 mm. in standard

length, was observed for a period of half an hour at the shore of the Ala Wai Yacht Basin. No fish became separated by more than 12 in. from the rest of the group.

The schooling of *A. triostegus triostegus* during spawning has been discussed previously.

Reaction to Danger

Juvenile manini along the shore of a part of the Ala Wai Canal infrequently visited by man perceive the approach of a person walking along the bank from an average distance of about 20 ft. This distance is contingent on the position of the sun and the depth and turbidity of the water. The usual reaction is a swift darting to some immediate hiding place. If an observer remains immobile, the fish come out of hiding in from about 15 to 25 sec. and resume feeding shortly thereafter. Manini of the same size next to the sea wall of the Ala Wai Yacht Basin, where people pass almost continuously, do not interrupt their feeding at the approach of a person unless some unusual sharp movement is made, at which time they may hide.

The manini in a large tide pool at Diamond Head were observed to have two hiding places, each beneath ledges at the side of the pool. One of these was preferred, and most of the fish swam to it when the pool was approached. When rotenone was spread in the region of this retreat, all of the fish swam rapidly in a direct line to the second ledge, in spite of the turbidity of the water (from the rotenone), even though they had to move in the direction of the observer and pass over a very shallow part of the pool. The part of the pool away from the observer was broader, deeper, free of rotenone at the time, but without good cover. It was evident that the fish were well aware of the location of the best places to seek refuge.

An experiment was designed to determine the time required by juvenile manini to recognize and accept a new site of cover. A small opaque plastic bowl was inverted and placed with one edge elevated from the bottom of a 23 gal. aquarium in which 12 manini, 26 to 39 mm. in standard length, had been kept without a place of shelter for 3 days. Initially this bowl was avoided by all the fish. Fifteen min. elapsed before any fish swam near it. One hr. after the bowl had been added to the tank, the manini were frightened by rapid arm movements all around the aquarium; however none sought refuge in the new shelter. Upon being similarly frightened 3 hr. later, 2 of the smallest fish went into the bowl. Three hr. after this, all of the manini swam to the bowl for cover.

Adult manini are less inclined to seek shelter. Their reaction to an approaching swimmer usually takes the form of retreat. When cover is sought, the hole into which the fish swim usually has two or more entrances. As has been pointed out by Breder (1949*b*: 97) a reaction to attack which alternates between flight and hiding is common among fishes.

Fighting

Juvenile manini may often be seen fighting among themselves in the natural environment. Also, several days after being placed in an aquarium, this behavior becomes noticeable. It increases with time to the extent that it interferes with feeding. After 2 weeks in an aquarium, fully half the diurnal time of 12 juveniles was spent at this activity. The fighting was not associated with territoriality, but took place among all the fish and in all parts of the tank. Three of the larger fish were consistently the aggressors, and the smaller fish usually bore the brunt of their attack, but no definite peck order was established. When only 2 fish were present, however, the larger invariably dominated the smaller and forced it to remain in a corner of the aquarium for much of the day.

A distinct color change, which results in an almost complete reversal of the usual color pattern, accompanies the fighting. The broad pale interspaces between the vertical black bars become very dark, and the upper seven-eighths of the bars change to yellowish white except for a fine dark line in the center of each. On the lower eighth of the body the black bars are faintly visible in their normal width. The more aggressive fish are darker than the less aggressive ones.

The fighting usually involves little actual contact between fish. Most of the time a fish under

attack hastily withdraws, a response which generally satisfies the attacker for the moment. When there is no immediate retreat the two fish circle at close quarters and make pugnacious movements, mostly with the posterior part of the body. When a new manini was placed in the tank, it was attacked with ferocity by all of the other manini, but rarely more than one at a time. The hapless fish was pecked over the head and body, strongly bumped, and lashed at with the caudal spines of the others as it scurried from one part of the tank to the next. A manini twice the size of any in the tank was given the same treatment. After about half hour of incessant attack, this oppressed fish was observed to lie on its side on the bottom and submit completely to the relentless abuse. It was caught and examined shortly thereafter. Only a few small scratches on the fins and two small cuts on the body could be seen. The manini has a much smaller caudal spine than other species of *Acanthurus* and is not capable of inflicting much damage with it. Also the jaws do not seem strong enough to injure other fishes of equal size.

Manini were never observed to fight with other species of fishes. They appear to display a dominant behavior pattern to at least some, however. Six small pomacentrids (*Abudefduf abdominalis*) ranging from an estimated 18 to 38 mm. in standard length were observed to intermingle with 4 juvenile manini about 28 to 36 mm. in standard length at the shore of the Ala Wai Yacht Basin. Although the pomacentrids swam close to one another, they usually avoided coming within a body length of any manini. When one did and this was observed by a manini, the latter responded by a slow sideward movement of the body toward the intruding fish. The posterior half of the manini's body was curved toward the pomacentrid and vibrated slightly. The pomacentrid invariably retreated a short distance.

Breder (1948: 293) observed similar behavior in an aquarium with *A. chirurgus*. He noted that a peck order was established among certain fishes in an aquarium. This involved 4 pomacentrids and 1 gerrid. A small specimen of *A. chirurgus* was added which was about equal in size to the largest pomacentrid. Although the surgeonfish was not bothered much by the other fish at first, it seemed to be low or lowest on the peck order. It was never noted to pursue the others, but was occasionally pecked at by the others. Fourteen days later the peck order was modified and the acanthurid was definitely number one. There was little fighting but all kept clear of the surgeonfish which "showed" its peduncular spine to the more timorous.

I have never observed fighting among adult manini. Larger individuals may display dominance over smaller ones, however.

Behavior at Night

At night, both in the natural habitat and in the aquarium, manini rest on the bottom, usually with the pelvic fins and anal fin touching the substratum, in what appears to be a state of torpor or sleep. In general the body color is darker than during the day. Large dark blotches which may be seen faintly during the day between the vertical dark bars are more conspicuous at night. This resting phase is not without movement, for the pectoral fins are almost in constant slow motion and there are vertical undulations of the median fins. When a light was flashed on a darkened aquarium at night, short movements over the bottom were occasionally found to be in progress. These were directed backward more often than forward.

Usually the manini in an aquarium or a tide pool at night are well dispersed. In the normal environment most of the fish choose a sheltered although not a confining location. Many, however, may be seen in very exposed locations. Breder (1948: 294) observed that *A. chirurgus* rested on the bottom of an aquarium at night in the proximity of some shells, but in no case did it enter or hide in the shells.

If light is turned on a sleeping manini at night, and no sharp movements are made, the fish can be lightly touched or even picked up without its making any effort to escape. If a sharp movement is made in the water of the aquarium, or if the fish is roughly handled, it will dart away for a considerable distance over the bottom. This behavior was put to good use for the collection of this species alive. Using a head lamp at night to locate a sleeping fish, a dip net was cautiously placed in front of the

quarry. A long stick was used to prod the posterior end of the fish which often responded by swimming into the net.

The following observations on sleep were made on 10 juveniles, 27 to 40 mm. in standard length, which had been maintained in an aquarium for 12 days.

With the onset of darkness the fish settled to the bottom of the tank, although they continued to move about, and occasionally these movements would take them above the bottom. After total darkness ensued, 25 min. were required before they entered the state of suspended animation completely. This state was ascertained by lightly stroking the fish with a glass rod. When they ceased to flee from such contact, it was assumed that they were asleep. Since the period of attaining sleep was prolonged by the interruptions, a series of observations on successive nights was necessary to determine the normal period.

The stimulus to sleep was shown to be the onset of darkness and not merely the passage of a definite number of hours of diurnal existence, for the manini remained active for many hours when a light was kept burning above the tank after darkness set in. In one experiment the fish were kept awake for a period of 24 hr. They remained active during the time; however they ceased to feed toward the end of this period and displayed "restless" swimming.

When the fish were kept in total darkness for 24 hr., they remained relatively quiet on the bottom during the entire period. At the end of this period, however, it was noticed that they perceived the immersion of a hand into the tank regardless of how slowly it was inserted.

After 3 hr. of uninterrupted sleep, beginning with onset of darkness, lights were turned on to see how long the state of torpor would persist. In 13 to 14 min. the first 3 fish awakened and began to swim around the aquarium. These were the largest of the 10 manini. The remaining fish awakened according to size; the 2 smallest required 25 and 28 min., respectively. All of the fish displayed very restless swimming, moving constantly back and forth in the aquarium. When the lights were turned off, they required 39 min. to re-enter the state of torpor. No distinction could be detected in the time needed for the different-size fish to go back to sleep.

SUMMARY

1. In the Hawaiian Islands the common surgeonfish, *Acanthurus triostegus sandvicensis* (known in Polynesia as the manini), is found in many different habitats, although never far from coral or rock for shelter and algae for food. The young are abundant in tide pools.

Manini are not numerous in regions of heavy coral cover, although certain other surgeonfishes are surprisingly common there.

2. The eggs and larvae of the manini are pelagic. Eleven acanthurid larvae, 4.2 to 8.7 mm. in length, were taken in eight plankton tows 10–140 mi. from the nearest Hawaiian island. All were from the upper 50 m., although more tows were made in from 100 to 300 m. A higher percentage of acanthurid postlarvae have been found in surface-caught yellowfin tuna than those taken with long line at depths of about 30 to 160 m. Acanthurid larvae are more commonly found in the stomachs of tuna caught inshore than offshore.

3. The limits of temperature tolerated for 1 hr. by manini (both young and adults) appear to be about 12° and 37° C. Chlorinity tolerance for 24 hr. approximates 1.4 ‰ to 37 ‰. The most extreme readings of temperature found on Oahu in waters inhabited by manini were 16.2° and 35.1° C. and of chlorinity, 2.65 ‰ and 21.3 ‰.

4. Apart from the postlarval acanthurids found in tunas nothing is known of predation on the pelagic stages of the manini. In Hawaii, juvenile manini probably constitute prey to carangid, muraenid, synodid, scorpaenid, sphyraenid, cirrhitid, holocentrid, and antennariid fishes, and possibly certain large crabs. In view of the paucity of large piscivorous fishes in Hawaiian waters adult manini are probably relatively free of predation (man excluded).

5. The manini was found to be parasitized by one species of hydroid, four trematodes, four nematodes, two leeches, and six copepods.

6. Difficulty was experienced at one time maintaining manini in aquaria. They died in

about 48 hr., displaying large sores, eroded fins, and excessive mucus on the body. The dominant bacterium was a short, gram-negative rod.

7. The stomachs of 3 postlarval manini 6.7 to 7 mm. in length were examined for food. Two were empty, and 1 contained two appendicularians and a larval polychaete. Of 57 manini in the acronurus (late postlarval) stage taken by night light offshore, 45 were empty, and the rest contained only occasional fish scales or crustacean fragments lodged in intestinal folds, thus suggesting that the acronuri do not feed at night. The guts of 4 unidentified acronuri taken from a skipjack stomach caught during the day were filled with zooplankton.

8. During the first day of transformation from the acronurus to the juvenile state, most manini do not feed at all. Feeding on algae becomes progressively heavier as transformation continues.

Juvenile and adult manini feed almost entirely on relatively fine filamentous algae of numerous species. Little inorganic sediment was seen in the gut contents. When 28 different kinds of algae plus diatoms were offered to manini in feeding experiments, a red alga (*Polysiphonia* sp.) and a green (*Enteromorpha* sp.) were preferred. All of the blue-greens, the red *Asparagopsis*, and one species of the brown alga *Ectocarpus* were not ingested (although these may be found in stomach contents, mixed with other algae).

9. Juvenile manini were maintained in aquaria on algae alone. Fish 26 to 31 mm. in standard length grew at the rate of about 6 mm. per month (temperature 23.0° to 23.8° C.) when fed either *Polysiphonia* sp. or *Enteromorpha* sp.

10. Manini feed almost constantly during the day, and a large volume of algae is passed through the gut. Four juveniles weighing a total of 8.3 grams consumed 10.8 grams of *Enteromorpha* in 1 day and 8.8 the next. Two adults weighing 83.2 and 94.5 grams ate a total of 27.4 grams of *Enteromorpha* in 1 day. The adult fish did not feed as frequently in aquaria as in the natural environment. The larger fish dominated the smaller one and consumed the major part of the algae.

11. Manini showed no response to extracts of algae, and the visual sense alone is needed to locate algal food. The olfactory sense appears to function in feeding only to avoid unsavory material. Chemoreceptor organs in the mouth and possibly tactile organs as well seem to be associated with the acceptance of algae and the rejection of material not utilized as food.

12. The morphology of the digestive system is briefly discussed. The alimentary tract increases nearly three-fold during the 4 or 5 days of transformation from the acronurus to the juvenile form, reflecting a change in food habits from feeding on zooplankton to feeding on algae. The length of the gut increases from about three times the standard length in small juveniles to nearly six times in large adults.

13. Algae passed through the gut of juvenile manini in 1 hr. 45 min. and in about 2 hr. in adults. At night when feeding ceases, the gut is emptied slowly.

14. Although sustaining growth, the *Enteromorpha* appeared little affected after passing through the gut of manini. Additional work is needed to determine what is utilized by the manini from its algal food.

15. The stomach of the manini varies from slightly acidic to slightly alkaline. The intestine is alkaline (pH 7.7 to 9.1) and the gall bladder acidic (pH 6.2 to 6.4).

Amylase was detected throughout the digestive tract, strongest in the pancreas and weakest in the stomach. The pH optimum of the amylase is about 6.7. Maltase was not found.

Lipase, with a pH optimum of 7.2, was found in all digestive organs, strongest in the pancreas and pyloric caeca and weakest in the stomach.

Proteolytic enzymes are weak, especially from the stomach; pH optimum of pancreatic protease is about 8.4.

16. About two-thirds of the adult manini caught in unbaited traps off Oahu in from 30 to 90 ft. are males. Of 221 manini in museum collections from the tropical Pacific mostly taken from inshore waters, 134 (60.7 per cent) are females.

17. Although ripe males may be taken throughout the year (fewer, however, in the fall), ripe female manini have been found in Hawaii only from early December to late July (and transforming young from middle February to early October). Average monthly sea surface tempera-

tures in the Hawaiian Islands vary from about 75° to 81° F. Enlargement of the gonads appears to coincide with decreasing water temperature in the fall.

18. An estimate of about 2½ months for the duration of larval life of the manini in Hawaii was made by noting the time between the finding of the first ripe females and first transforming young of the season and the last ripe fish and last incoming acronuri.

19. Ripe female manini (*Acanthurus triostegus triostegus*) were found in collections made at islands of the tropical Pacific (where mean sea surface temperatures exceed 80° F. throughout the year) in all months of the year, as were transforming or early juvenile specimens, thus indicating year-round spawning in more equatorial waters.

20. A lunar periodicity in the number of transforming manini was discovered from tidepool collections on Oahu, with the peak in spring occurring a few days before new moon. Since a collection of young from the Gilbert Islands, where water temperatures average about 7° F. higher (and hence development is probably more rapid), indicates peak influx of acronuri approximately at the time of full moon, the periodicity is not attributed to any lunar effect on the young but to greater spawning by adults at one time of the month than another. Thirty-nine ripe female fish were found among 2,552 adult manini examined from 12 days before to 2 days after full moon during the spawning season and 4 among 1,311 fish seen in the remaining half of the lunar month.

21. Individual manini appear to spawn more than once during the season. Large and small ripe females were found near the start and end of the season. No completely spent ovaries were observed until July and August. In graphs of egg diameters of mature ovaries, a mode of egg size about intermediate to the diameter of transparent ripe eggs and primordial ova was invariably present during the season.

No specimens were found with modes of egg size lying between the intermediate-size mode and that of fertilizable eggs, thus suggesting that ripe eggs develop rapidly from the opaque ova of the intermediate group.

22. Although the spawning of manini was never witnessed in the Hawaiian Islands, *A. triostegus triostegus* was observed to spawn in the Tuamotu Archipelago. Several hundred fish were seen in a milling aggregation at dusk during the time of full moon. Many had broader bars on the body and darker fins than normal; 5 of these were speared and proved to be ripe males. Two normally colored manini were females, 1 running ripe. Spawning occurred at the apex of sudden rapid upward movements by small groups of fish. A current carried the eggs to the open sea.

23. The smallest running ripe female manini which was found on Oahu was 101 mm. in standard length; the smallest male, 97 mm. Manini probably do not spawn in their first year.

24. The number of ripe eggs in the ovary of one 123 mm. manini was estimated at 40,000.

25. Early development was studied following artificial fertilization of the eggs. The eggs average 0.67 mm. in diameter, have a single oil globule, and float at the surface. Hatching occurs in 26 hr. at 24° C. As yolk is used up, the larvae show a progressive tendency to sink. They combat this by upward swimming movements. Feeding begins at the age of 5½ days. No young were maintained in aquaria more than 6½ days; however, 3 postlarval manini, 6.6 to 7 mm. in total length, could be identified from plankton collections by fin-ray counts. The most striking feature of their morphology is very elongate second dorsal, second anal, and pelvic spines.

26. Manini acronuri come into shoal water to transform only at night. They are discoid, scaleless, and transparent with a silvery abdomen. Their second dorsal, second anal, and pelvic spines are poisonous. During transformation, which requires 4–5 days, adult-type configuration and coloration is attained, scales form, and the venomous quality of the spines disappears.

27. Light appears to be essential to transformation. Two acronuri taken at midnight were placed in a darkened 2½ gal. battery jar. They failed to transform, whereas 3 others in a comparable but not darkened container completed their metamorphosis to juvenile form.

28. A variation in standard length from 22 to 29.5 mm. was noted in 175 manini acronuri from Oahu in their first day of transformation. One hundred and sixteen were collected in tide

pools from 5 days before to 5 days after peak tide-pool recruitment (2 days before new moon). When the lengths of these manini were compared with the lengths of the 59 acronuri from the rest of the lunar month, a smaller range of length (23.5–28.5 mm.) was apparent for the former group in spite of nearly twice as many specimens. Presumably individual acronuri are capable of transforming some days before the rest of their age group, and hence at a smaller size, when they come inshore earlier than the others. Similarly, those which reach shoal waters later than the majority will have attained larger than average size.

29. A variation in size at transformation was found with locality which appears related to water temperature. The smallest size occurs in the warmest regions (about 20 mm. in the East Indies) and the largest in cooler sectors (Phoenix Islands excepted).

In the Hawaiian Islands the size at transformation averaged 26.3 mm. for 39 specimens collected in April and May and 25.4 mm. for 38 specimens which were taken from July to October (thus developing during warmer months).

30. Growth of juvenile manini, as determined by rearing of captive fish, recovery of fish marked by clipping rays from the top or bottom of the caudal fin, and progression of modes in successive graphs of tide-pool collections on Oahu is about 12 mm. per month.

The recovery of 281 manini of 1,247 tagged with Petersen disk tags fastened with monofilament nylon provided information on the growth of larger fish. The growth of adults 100 to 120 mm. in standard length is slightly greater than 1 mm. per month; 120 to 141 mm. fish grow about 0.8 mm. per month.

Growth of both juveniles and adults ceases during the winter months in the Hawaiian Islands.

31. No annular marks could be detected on the scales, otoliths or vertebrae of manini from which estimates of age could be made. Two larger acanthurids, *Naso unicornis* and *Acanthurus xanthopterus*, lived 20 and 15 years respectively in the Waikiki Aquarium in Honolulu where they died as the result of accidents.

32. Small juvenile manini tend to remain in the tide pool or shallow water area to which they first come as acronuri. With increasing size they move seaward.

Locality data from the recovery of tagged adult manini was too general to determine local movements, but adequate to demonstrate that no extensive migrations are normally undertaken by this surgeonfish. If displaced from their home area, however, manini are capable of wandering distances as great as 8 mi.

33. Manini may be seen as solitary fish, in small groups, or in large feeding aggregations. One large group of fish, although observed to remain in one region in the Tuamotus, moved as much as 300 yd. in several hours.

34. Juvenile manini in a large tide pool on Oahu were observed to have two hiding places beneath ledges, one of which was preferred. In order to attain shelter they will swim toward an intruder, if necessary. From 4 to 7 hr. were required for aquarium fish to accept an inverted bowl tilted on edge as shelter.

Adults are less inclined to seek shelter. Their reaction to an approaching swimmer usually takes the form of retreat.

35. Juvenile manini frequently fight among themselves, both in the natural environment and after being adapted to aquarium life. This does not concern territoriality, but is an expression of dominance by larger fish over smaller fish or old residents (in the case of aquarium fish) over new arrivals. A distinct color change, which involves an almost complete reversal of color pattern, is readily apparent, with the more aggressive fish becoming the darkest. Generally there is no contact between fish, and the fish under attack usually retreat. If not, there is a circling at close quarters with occasional overt movements, especially with the posterior part of the body. The manini's caudal spine is small, and the juveniles were observed to inflict at most only superficial scratches.

Manini were not observed to fight with other species but they may display a dominance in their behavior towards some.

36. With the onset of darkness juvenile and adult manini settle to the bottom, usually with pelvic and anal fins in contact, and enter a state of torpor or sleep. Occasional short movements are made, these more often directed backward than forward. Even when remaining in one place

the pectoral fins are kept in slow motion and there are vertical undulations of the median fins. The color is darker than during the day, and large dark blotches are conspicuous between the vertical black bars on the body. A period of 25 min. after darkness ensued was found to be necessary for the fish to reach a state when they could be lightly touched without fleeing. When lights were kept on in an aquarium for 24 hr., manini remained active, however they ceased to feed toward the end of this period and swam restlessly. A light was turned on after 10 manini, 27 to 40 mm. in standard length, were allowed to sleep for 3 hr. From 13 to 28 min. were required for the fish to awaken, the smallest taking the longest time.

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A ZOOGEOGRAPHICAL ANALYSIS OF SPECIES OF *CYPRAEA* IN THE HAWAIIAN ISLANDS

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Introduction

The purpose of this paper is to give a brief account of the species of the mesogastropod genus *Cypraea* in the Hawaiian Islands, and to present a tentative analysis of its geographical components. The data are based in so far as possible on collections of living specimens made during the past thirty years by shell collectors in the Hawaiian Islands, and on material in the Bernice P. Bishop Museum in Honolulu, Hawaii.

The Hawaiian Islands are of particular interest in studies of the zoogeography of the Pacific because of their isolated position in the eastern Pacific, and because they are on the north-eastern periphery of the range of many Indo-Pacific organisms. Surrounded by deep water, the Islands' nearest neighbour is Johnston Island, 450 miles to the south-west (see Fig. 1). To the south are the Line Islands, at a distance of some 1,000 miles; to the east is the coast of North America, 2,000 miles away; and to the north-west is Wake Island, a northern outlier of the Marshall Islands, 1,200 miles from the northernmost island in the Hawaiian archipelago. The Hawaiian Islands form a narrow chain extending 1,500 miles south-east to north-west, from 18° N. latitude to 28° N. latitude. They are of volcanic origin; the north-western islands, which are the oldest, comprising low coral atolls, while the more recent eastern islands exhibit characteristic volcanic attributes. The predominant winds are the north-east trades.

Cypraea from the Hawaiian Islands

The following list of species, which appear in alphabetical order, is supplemented by brief notes on habitat and distributional records. Comments on nomenclature have been included where clarification was thought necessary. "Beached" specimens refer to those specimens which have been washed ashore. The general distribution record is that of Schilder and Schilder (1938-1939); specific distribution records for the Pacific are based on specimens checked by the author. The term "Eastern Hawaiian Islands" refers to the south-eastern group of islands, comprising Niihau, Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii, as distinguished from the north-western or leeward islands (see Fig. 1).

C. arenosa Gray

Habitat.—At depths of 6 to 35 feet in coral heads, the animals usually deep in the interstices of coral or at the base of coral trunks.

Distribution.—Pacific Ocean (Schilder and Schilder, 1938-1939). Recent collections: Marshall Islands, Tuamotu Islands, Line Islands, Johnston Island. Throughout the Hawaiian Islands.

C. caputserpentis Linn.

Habitat.—Shoreline, inshore waters to a depth of 15 feet; one living juvenile dredged from 350 feet. The most abundant of the shallow-water species in Hawaii.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. carneola Linn.

Habitat.—At depths around 30 feet in barren areas among dead coral; occasionally in shallow waters of inshore reefs. Rare as living animals; common as fossils in Pleistocene limestone of Oahu.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands only.

C. cernica Sowerby

Habitat.—Living specimens not known from the Hawaiian Islands; several shells dredged from waters off the eastern Islands in 1959; beached specimens from Midway.

Distribution.—Indo-Pacific—Mauritius and Chago, New Caledonia, and the Loyalty Islands (Schilder and Schilder, 1938–1939). Recent collections: Baker Island (Bernice P. Bishop Museum specimen). Presumptively throughout the Hawaiian Islands.

C. childreni Gray

Habitat.—One sub-fossil from Honolulu Harbor dredgings; three beached specimens from Midway. No living specimens known from the Hawaiian Islands.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Recent collections: Line Islands, Marshall Islands, Tuamotu Islands, Johnston Island. Presumptively throughout the Hawaiian Islands.

C. chinensis Gmelin

Habitat.—At depths of 30–60 feet in coral heads. Beached specimens occasionally found. Rare: six to eight living collections only.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Recent collections: Marshall Islands, Line Islands. Eastern Hawaiian Islands only.

C. cicercula Linn.

Habitat.—At depths of 30–60 feet in the interstices of coral heads. Beached specimens common in certain areas: Paumaluu, Oahu; Milolii, Kauai.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands only.

C. erosa Linn.

Habitat.—One known living collection under coral in 6 feet of water (Burgess, personal communication). Frequent as a fossil in Pleistocene limestone on Oahu.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands only.

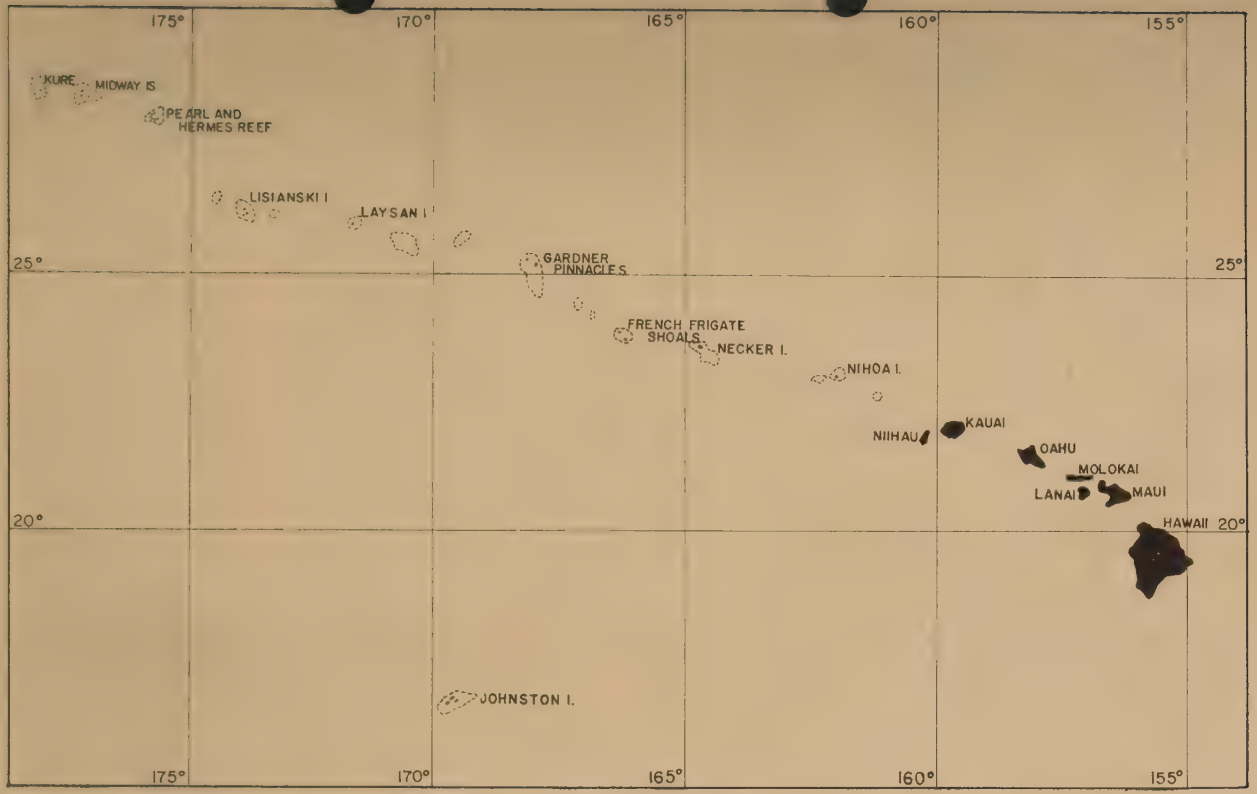

FIG. 1.—Chart of the Hawaiian Islands. From U.S. Coast and Geodetic Survey Chart 4000, 6th edition.

C. fimbriata Linn.

The shells described by Schilder (1933) as *C. waikikiensis* were apparently beached specimens. When Hawaiian specimens of *C. fimbriata* are compared, no conchological differences can be detected, nor are there any apparent radular differences from shells which might conceivably fit Schilder's description. *C. waikikiensis* is therefore considered here as a synonym of *C. fimbriata* Linn.

Habitat.—Shoreline, inshore waters, and to depths of 60 feet. Frequent.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. gaskoini Reeve

Habitat.—At depths of 30–60 feet in coral heads. Fairly common as beached specimens. Living specimens occasional.

Distribution.—Apparently endemic to the Hawaiian Islands. Throughout the Island chain except for Midway.

C. granulata Pease

Habitat.—At depths of 10–45 feet in coral heads ; occasionally in shallow water.

Distribution.—Apparently endemic to the Hawaiian Islands. Throughout the Hawaiian chain. Johnston Island.

C. helvola Linn.

Habitat.—Shoreline and inshore waters to depths of 60 feet.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. isabella Linn.

C. controversa Gray is here considered a synonym of *C. isabella* Linn., as no anatomical differences in mantle, reproductive structures, or radula have been detected. When specimens are measured, their length frequencies form a bell-shaped curve, with no indication of bimodality which might be expected if two species were being considered.

Habitat.—Shoreline and to depths of 60 feet ; dredged specimens from 350 feet. Abundant.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian chain.

C. leviathan Schilder-Schilder

Although Allan (1956) has suggested that because of the variability exhibited in shell characters, *C. leviathan* should be considered a synonym of *C. carneola* Linn., *C. leviathan* is here considered a distinct species. Anatomical evidence for this view will be published later.

Habitat.—Shoreline and to depths of 60 feet. Abundant.

Distribution.—E. Polynesia (Schilder and Schilder, 1938-1939). Recent collections : Tuamotu Islands, Marshall Islands, Johnston Island. Throughout the Hawaiian Islands.

C. lynx Linn.

Habitat.—Two known living collections from inshore waters (Burgess, personal communication). Common as a Pleistocene fossil and as a subfossil in recent reef dredgings.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. maculifera Schilder

Habitat.—Shoreline in crevices and under wave-swept benches; to depths of 60 feet.

Distribution.—Pacific Ocean (Schilder and Schilder, 1938–1939). Recent collections: Marshall Islands, Line Islands, Tuamotu Islands. Throughout the Hawaiian Islands.

C. mariae Schilder

Habitat.—Beached specimens only (Paumaluu, Oahu).

Distribution.—Pacific Ocean (Schilder and Schilder, 1938–1939). Recent collections: Line Islands, Marshall Islands. Eastern Hawaiian Islands (Oahu) only.

C. mauritiana Linn.

Habitat.—Shoreline, usually in basalt crevices; to depths of 30 feet. Common.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands and Necker and Nihoa.

C. moneta Linn.

Habitat.—Shoreline, in tidepools and under rocks in shallow, sandy areas.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. nucleus Linn.

Habitat.—One living specimen from inshore waters (Burgess, personal communication). Beached and fossil specimens from Oahu.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands (Oahu) only.

C. ostergaardi Dall

Habitat.—Living specimens not known; shells dredged from 350 feet; beached specimens frequent.

Distribution.—Apparently endemic to the Hawaiian Islands. Throughout the Hawaiian chain.

C. poraria Linn.

Habitat.—Shoreline and inshore reefs to depths of 60 feet.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Eastern Hawaiian Islands only.

C. rashleighana Melvill

Habitat.—At depths of around 30 feet in coral heads; dredged from 350 feet. Beached specimens common.

Distribution.—Pacific Ocean—Loyalty Islands and New Caledonia; Hawaiian Islands (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. scurra Gmelin

Habitat.—At depths of 6–25 feet in coral heads. Frequent.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. semiplota Mighels

Because of the variation in shell size, various suggestions have been made that two species should be considered. Radular studies show no differences among specimens of different size ranges, and size frequency histograms show a bell-shaped curve.

Habitat.—Although living specimens were commonly collected in shallow inshore waters in branched coral up to twenty years ago, there has been no known living collection within the past ten years. Beached shells common on all islands.

Distribution.—Apparently endemic to Hawaii. Throughout the Hawaiian Islands.

C. sulcidentata Gray

Habitat.—At depths of 30–60 feet in coral heads; occasionally in shallow waters; dredged from 350 feet.

Distribution.—Apparently endemic to the Hawaiian Islands. Throughout the Hawaiian Islands.

C. talpa Linn.

Habitat.—At depths of about 10 feet in inshore waters; in coral at 30–60 feet. Frequent.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938-1939). Throughout the Hawaiian Islands.

C. teres Gmelin

Habitat.—Shoreline and inshore waters to depths of 100 feet. Common.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. tessellata Swainson

Habitat.—At depths of 30–60 feet; dredged from 350 feet.

Distribution.—Apparently endemic to the Hawaiian Islands. Throughout the Hawaiian Islands.

C. tigris Linn.

Habitat.—At depths of 10–100 feet. Common.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

C. vitellus Linn.

Habitat.—Inshore waters, and occasionally on shoreline; common twenty years ago but collectors reported only four or five specimens within the last five years.

Distribution.—Indo-Pacific (Schilder and Schilder, 1938–1939). Throughout the Hawaiian Islands.

Components of the Hawaiian Cypraeid Fauna

The Hawaiian cypraeid fauna is most conveniently divided into two components: (1) an Indo-Pacific element which includes twenty-five species, twenty of which have a recorded distribution from East Africa through the Indo-Pacific to Hawaii, and five of which have been recorded from the Pacific Ocean only; and (2) an endemic element of six species. All species, with the exception of four, have been recorded throughout the Hawaiian chain (Table 1). Ten of the Indo-Pacific species have also been recorded from Clipperton Island, off the coast of California; none of these, however, has been collected alive, although shells of four species have been described as " fresh " (Hertlein and Allison, 1960).

The Hawaiian cypraeid fauna apparently comprises an attentuated Indo-Pacific fauna. Distribution records from other Pacific island groups indicate a greater number of species occurring to the west and south of the Hawaiian Islands. Forty-eight species have been recorded from the Marianas Islands (Ward, 1959); and approximately forty species are known from both the Marshall Islands and the Line Islands. Although comprehensive collections from Wake Island and from Johnston Island are not yet available, several Indo-Pacific species are recorded from those islands which do not occur in Hawaii.

The distributional records of two species within the Pacific present some puzzling features. *C. cernica*, which is recorded by the Schilders (1938–1939) from Japan and the Loyalty Islands, and which is represented in the Bernice P. Bishop Museum in Honolulu by one specimen from Baker Island (Phœnix Islands), has not been recorded elsewhere except for several recently dredged shells from Hawaiian waters, and a beached specimen from Midway. *C. rashleighana* is recorded by the Schilders (1938-1939) from the Loyalty Islands and New Caledonia, in addition to the Hawaiian records of the species. While it is possible that the meagre records of these two species reflect lack of collecting activity, they may possibly represent a relict fauna.

Anatomical and conchological studies of the endemic Hawaiian species indicate that they are apparently derived from the Indo-Pacific component of the cypraeid fauna. *C. isabella* and *C. nucleus*, both of which have a wide Indo-Pacific distribution, are related to *C. tessellata* and *C. granulata* in Hawaii; and *C. arenosa* which occurs in the Pacific to *C. sulcidentata* (Kay, 1960). It is suggested on the basis of radular and conchological studies that *C. cribraria*, which is distributed throughout the Indo-Pacific, is the ancestor of the Hawaiian endemic *C. gaskoini*, and *C. staphylaea*, which is also of wide Indo-Pacific distribution has given rise to *C. semiplota* in Hawaii. The postulated derivation of *C. ostergaardi*

TABLE I

Cypraea	North-western Islands								Eastern Islands				
	Kure	Midway	Pearl and Hermes	Lisianski	Laysan	French Frigate Shoals	Necker	Nihoa	Kauai	Oahu	Molokai	Maui	Hawaii
arenosa						×			×	×	×	×	×
caputserpentis		×	×	×	×	×	×	×	×	×	×	×	×
carneola									×	×		×	×
cernica		×								×			
childreni		×								×			
chinensis									×	×			
cicercula		×							×	×			
erosa									×	×			
fimbriata	×	×	×			×			×	×	×	×	×
gaskoini					×				×	×	×	×	×
granulata	×	×	×	×	×	×	×		×	×	×	×	×
helvola	×	×	×	×	×	×		×	×	×	×	×	×
isabella	×	×	×	×	×	×	×	×	×	×	×	×	×
leviathan						×			×	×	×	×	×
lynx		×							×	×			
maculifera					×	×		×	×	×	×	×	×
mariae									×	×			
mauritiana		×					×	×	×	×	×	×	×
moneta										×	×	×	×
nucleus		×							×	×			
ostergaardi									×	×			
poraria		×	×						×	×	×	×	×
rashleighana									×	×			
semiplota	×	×	×		×				×	×	×	×	×
scurra	×	×		×	×	×			×	×	×	×	×
sulcidentata	×	×	×	×	×				×	×	×	×	×
talpa				×	×				×	×	×	×	×
teres	×	×	×		×	×			×	×	×	×	×
tessellata	×	×	×	×	×				×	×	×	×	×
tigris	×								×	×	×	×	×
vitellus		×	×						×	×	×	×	×

from *C. helvola* (Ostergaard, 1928) must remain tentative until living specimens of *C. ostergaardi* are available for study.

Frequency, Habitat, and Size Variation

When the species of *Cypraea* which occur in Hawaiian waters are compared with their counterparts elsewhere in the Pacific, three points emerge : (1) there is a shift in the " dominant " species among island groups, with some of the more commonly occurring species of the central Pacific of such rare occurrence in Hawaii as to make them negligible components of the cypraeid fauna of the Islands at the present time ; (2) at least two species have an apparently different habitat in the Hawaiian Islands than they have elsewhere in their range ; and (3) Hawaiian specimens of

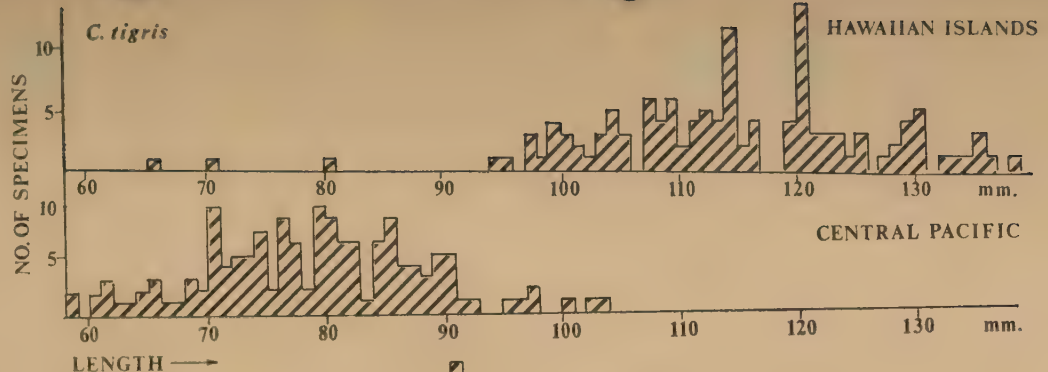

Fig.2

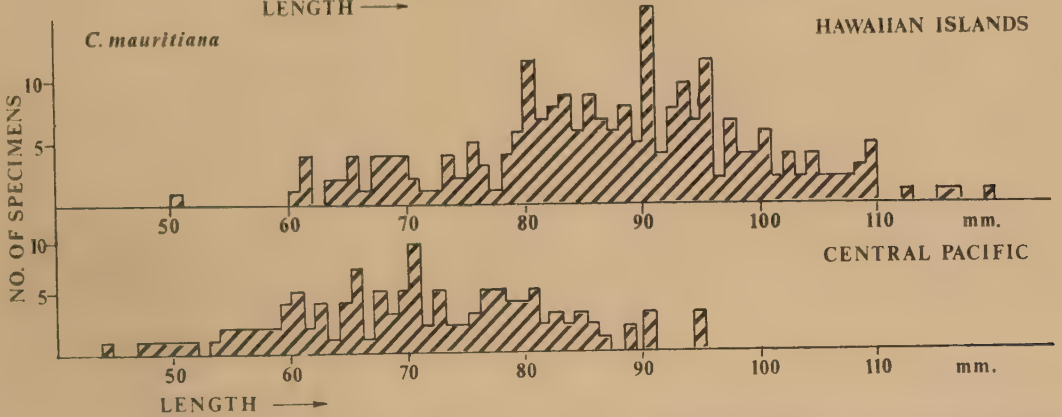

Fig.3

Fig. 2.—Length-frequency histogram of *C. tigris* in the Hawaiian Islands and the Central Pacific.
Fig. 3.—Length-frequency histogram of *C. mauritiana* in the Hawaiian Islands and the Central Pacific.

six species are larger than specimens of the same species from other Pacific Island groups.

The most abundant shallow-water species in the Hawaiian Islands is *C. caputserpentis*, thirty to forty specimens of which have been collected from quadrats 100 feet square on algal-covered benches in one hour's collecting time. The only other shallow-water species which has been collected in large quantities is *C. maculifera*, lots of fifteen to twenty specimens of which have been collected at depths of up to 15 feet. In deeper waters both *C. scurra* and *C. isabella* have been collected in lots of up to fifty specimens.

Of those species described as " abundant " or " extremely common " on other Pacific islands, *C. moneta* is most frequently mentioned (Ingram, 1938 ; Bayer and Neurohr, 1946 ; Ingram and Kenyon, 1945). Collections of *C. moneta* from Kapingamarangi, Caroline Islands, indicate that 150–200 specimens occur in small quadrats on both the lagoon and seaward

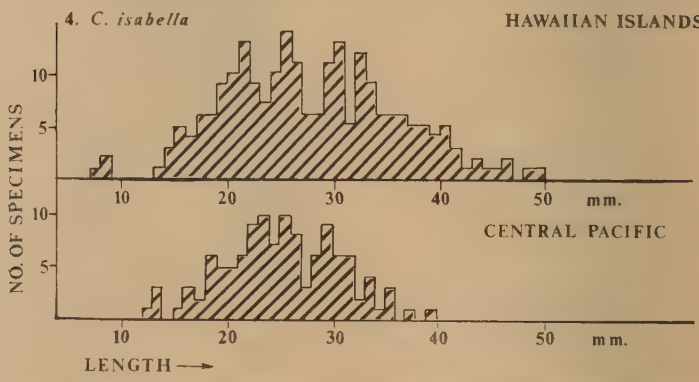

Fig. 4.—Length-frequency histogram of *C. isabella* in the Hawaiian Islands and the Central Pacific.

reefs. In the Hawaiian Islands two or three specimens of *C. moneta* may be collected at any one time in high tidepools or under rubble in sandy areas of the inshore reefs.

Two species which have been cited as " indicator species " elsewhere, *C. tigris* on the Great Barrier Reef (Stephenson, *et al.*, 1934), and *C. arenosa* in the Tuamotus (Doty and Morrison, 1954) occur in Hawaii, but in apparently different habitats from those they occupy elsewhere, and in far lesser abundance. *C. tigris*, described as exposed on the surface of large coral heads at Biak in the Marshall Islands (Bayer and Neurohr, 1946), and on various lagoon and seaward reefs in the Pacific (Demond, 1957), is collected only at depths below 10 feet in Hawaiian waters. *C. arenosa* which is collected on top of reefs in the Tuamotus (Doty and Morrison, 1954) occurs at depths of 6–35 feet in Hawaii.

Other species which have been noted as " common " elsewhere in the Pacific include *C. lynx* in the Marshall Islands (Bayer and Neurohr, 1946),

in the Admiralty Islands (Ingram and Kenyon, 1945), and in Tonga (Ostergaard, 1935); *C. erosa* in Tonga (Ostergaard, 1935); and *C. vitellus* in the Marshall Islands (Bayer and Neurohr, 1946) and in Tonga (Ostergaard, 1935). In Hawaii *C. lynx* and *C. erosa* have been collected as living specimens on only one or two occasions (Burgess, personal communication), although Ostergaard (1928) has pointed out that both species are fairly common as fossils in raised coral reefs of the Pleistocene on Oahu. *C. vitellus*, which also occurs as a fossil, is occasionally collected in Hawaii in shallow water.

At least six species are distinguished from their counterparts elsewhere in the Pacific by the larger size of the Hawaiian material. Size differences,

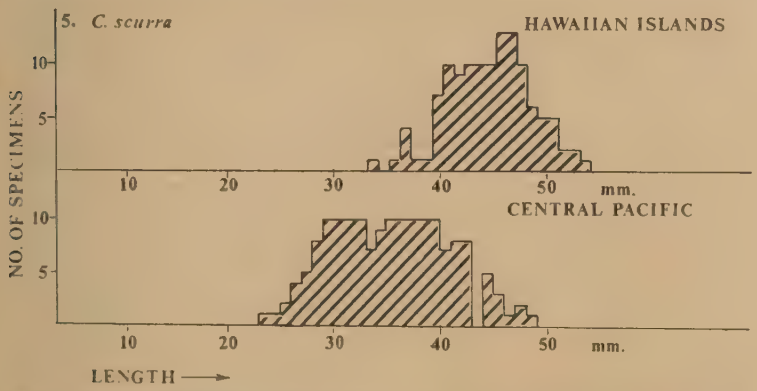

FIG. 5.—Length-frequency histogram of *C. scurra* in the Hawaiian Islands and the Central Pacific.

here manifested by length, are shown in Figs. 2-7. As is shown in Fig. 2, with one or two exceptions, the smallest examples of *C. tigris* in Hawaii are near the maximum reported size for the species elsewhere.

Because large size has frequently been noted in marine poikilotherms which inhabit colder waters (Bergman's law), specimens of those species which exhibit a larger size in Hawaii were compared with specimens of the same species from areas in similar latitudes to that of Hawaii. Specimens from the Bonin Islands and Japan (20° N. latitude) and from Tonga and Mangareva (20° S. latitude) do not apparently exhibit the same size phenomenon noted in the Hawaiian specimens, although sufficient data for a statistical analysis is not yet available; these specimens, rather, appear to fall well within the size range of species from Pacific Island groups which are close to the equator.

The shift in the "dominant" species among Pacific Island groups, the difference in habitat observed in Hawaii, and the greater size associated with some of the Hawaiian species may be correlated with ecological peculiarities of the Hawaiian habitat. While it is not the purpose of this paper to discuss these features in detail, it can be mentioned that they

primarily include differences in temperature and reef structure. In the latter instance the reefs of the eastern Hawaiian Islands lack several genera of reef-building corals such as *Acropora* which contribute to reef formation elsewhere in the Pacific. The bearing of these features, and

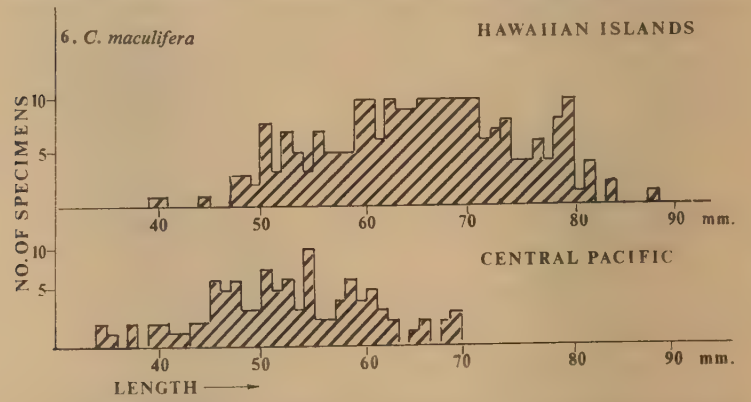

Fig. 6.—Length-frequency histogram of *C. maculifera* in the Hawaiian Islands and the Central Pacific.

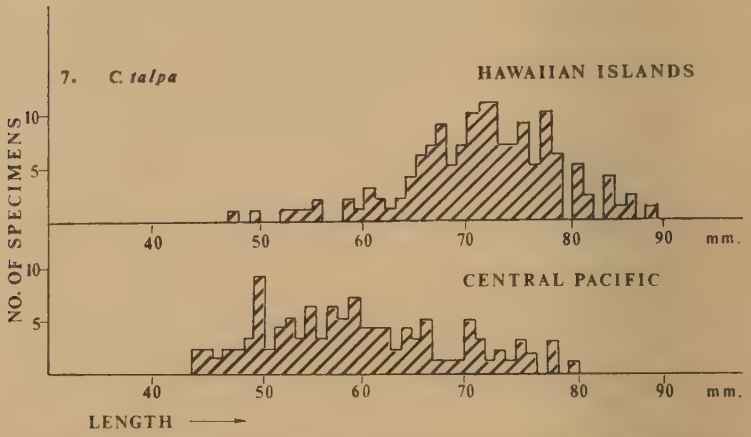

Fig. 7.—Length-frequency histogram of *C. talpa* in the Hawaiian Islands and the Central Pacific.

possible modes of speciation in the Hawaiian *Cypraea* which are suggested by the data described above will be discussed in a later paper.

Derivation of the Hawaiian Cypraeid Fauna

The predominance of taxa characteristic of the central Pacific, the differentiation of the endemic species from central Pacific stock, and

the absence of species characteristic of the Pacific coast of North America indicate the origin of the Hawaiian cypraeid fauna from the Indo-Pacific faunal centre. Because of the isolated position of the Hawaiian Islands, immigration into the islands was and is most probable by the pelagic larval stages known in most species of *Cypraea*. It is also possible that some organisms entered the Island chain attached to floating logs or other objects, as several species of *Cypraea* have been recorded from Pearl Harbor where they were scraped from the bottoms of ships (Burgess, personal communication).

There remain the problem of the direction of immigration into the Hawaiian Islands and a consideration of the role of the " neighbouring " islands as possible way-points into the Hawaiian chain. Analysis of the cypraeid species of the Line Islands and Johnston Island has not provided any evidence of movement from these localities into Hawaii. There is some evidence, however, that several species of *Cypraea* entered via the north-western or leeward islands of the chain, which implies a relationship with the fauna of the western Pacific Ocean.

That at least some species of *Cypraea* entered the Hawaiian archipelago by way of the north-western islands is suggested by the distribution of all endemic species, with the exception of *C. gaskoini*, throughout the Hawaiian group. Other explanations for the occurrence of the endemics throughout the chain are, of course, possible, e.g. that since they represent offshoots of the first immigrants there has been a long enough period of time for speciation to occur, there has also been sufficient time for spreading in the archipelago. Faunal records for other marine invertebrates, especially those for *Acropora*, a reef-forming coral at Midway which is absent from the eastern islands ; the records of several species of recently dredged molluscs from Hawaiian waters which resemble those of Japan ; and the similarity of several echinoderms of the north-western islands with those of Japanese waters (Fisher, 1925) are further evidence in favour of the hypothesis.

While it has not been reasonable to postulate any movement of *Cypraea* from the Line Islands or Johnston Island into Hawaii, there is some indication of movement from the Hawaiian Islands into Johnston Island. The record of *C. granulata* from Johnston Island, and the observations that specimens of *C. tigris*, *C. scurra*, and *C. maculifera* from Johnston fall within the size range of these species from the Hawaiian Islands, suggest that the Hawaiian cypraeid fauna has moved south-westward as far as Johnston Island. Clarke (1949) has noted also a relation between the echinoderm fauna of Johnston Island and the Hawaiian Islands ; and, more recently, Gosline (1955) reported a similar resemblance in the fish fauna, recording several species of Hawaiian endemics from Johnston Island.

Summary

Thirty-one species of the mesogastropod *Cypraea* are recorded from the Hawaiian Islands, twenty-five of which are distributed within the Indo-Pacific, and six of which are apparently endemic to the Hawaiian Islands. When we compare the Hawaiian cypraeid fauna with the

cypraeids of other Pacific Island groups, a shift in the "dominant" species among islands, differences in habitats of two species, and size variation in six species are apparent. Evidence for the derivation of the Hawaiian cypraeid fauna from the Indo-Pacific faunal centre is presented, and it is suggested that several species of *Cypraea* have entered Hawaii via the north-western or leeward islands of the chain.

Acknowledgment

I am greatly indebted to Dr. C. M. Burgess of Honolulu, Hawaii, for the use of his collection, and for much of the information which has been summarized in this paper. I would also like to thank Mr. Harold Jewell of Honolulu, Dr. Yoshio Kondo of the Bernice P. Bishop Museum in Honolulu, and the staff of the Museum for their help.

Contribution No. 150 of the Hawaii Marine Laboratory.

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ADDENDUM : Oceanographic data recently compiled by the United States Fish and Wildlife Service, Bureau of Commercial Fisheries, Hawaii Area, lend support to the hypothesis put forward in this paper concerning the direction of immigration of species of *Cypraea* into the Hawaiian chain. For the greater part of the year the Hawaiian Islands lie in a water mass which originates in the Western Pacific Ocean, and the general trend in the current system is from the north-west to the south-west, which would account for the Johnston Island findings.

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ANATOMICAL CHARACTERS WHICH DISTINGUISH TWO SPECIES OF *CYPRAEA*

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(*Read* 10 *June* 1960)

Introduction

Cypraea leviathan Schilder-Schilder was first described as a sub-species of *C. carneola* Linn. by Schilder and Schilder (1937), and later was raised to specific rank by the Schilders (1938–1939). The distinguishing features of *C. leviathan* when contrasted with *C. carneola* are its enormous size, emarginate anterior end, wider mouth, shallow fossula, pale, flesh-coloured, banded dorsum on which no or only a flesh-brown ring is present, and weaker, marbled sides. Allan (1956), noting the variability in size in the *carneola-leviathan* complex, has questioned the advisability of considering *C. leviathan* a distinct species, suggesting that it should be considered a sub-species of *C. carneola*. The purpose of this paper is to discuss certain anatomical features of specimens of extremes of the size range which tend to confirm the Schilders' distinction between *C. carneola* and *C. leviathan*.

Materials and Methods

The following descriptions are based on animals collected in the Hawaiian Islands, and are supplemented by radular descriptions of specimens from Kenya, East Africa, the Gulf of Akaba, Mauritius, the Caroline Islands, and the Society Islands. For purposes of species identification, specimens less than 45 mm. in length were identified as *C. carneola*, and those more than 55 mm. in length as *C. leviathan*.

Anatomical Characters

Radulae of specimens of extreme size ranges in the *carneola-leviathan* complex are distinctive in their general shape and texture, and in the structure of the individual teeth. The radula (Fig. 1) of specimens in the 55 mm. length range which conform to the Schilders' descriptions of *C. leviathan* is a heavy, solid structure in which the teeth are distinctly spaced in each radular row. The radula stains readily by various staining techniques such as the oxidation-dahlia technique of Bowell (1924). The outer marginal teeth (M 1) are horizontally elongate structures, the central cusp forming a shelf which extends the length of the tooth. The inner marginal teeth (M 2) and the admedians (AD) are rectangular structures with a prominent central cusp extending to the base of each tooth. The inner marginal teeth are distinguished from the admedians in that they are slightly wider than the admedians. The median (MD) is semicircular, with deeply excavated triangular cusps, the central cusp more prominent than the lateral cusps.

The radula (Fig. 2) of specimens in the 45 mm. length range which conform to the Schilders' descriptions of *C. carneola* is a light, almost

transparent structure which does not readily stain by the usual radular-staining techniques. The teeth are close together, overlapping each other on the radular ribbon. The outer marginals (M 1) are concave blades, a long median cusp forming the blade which is bordered by a short lateral cusp at the base of the blade. The inner marginals (M 2) are similar in general shape to the outer marginals, but the central cusp and body of the tooth are wider. The admedians (AD) are rectangular, with a broad base and three cusps, a large central cusp bordered by laterals. The median tooth (MD) is similar in shape to that of *C. leviathan*, being a semicircular structure with triangular cusps.

Although mantle characters and colour apparently differ within species

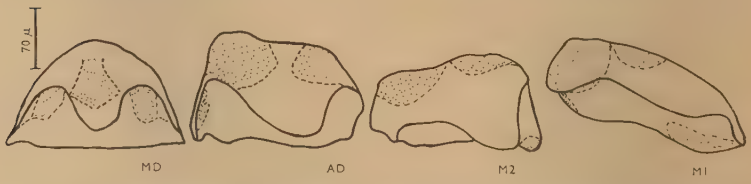

FIG. 1.—*Cypraea leviathan*. Half-row of radular teeth. Teeth spaced as on radular ribbon. Stippled areas indicate heavy staining. AD, admedian ; M 1, outer marginal ; M 2, inner marginal ; MD, median.

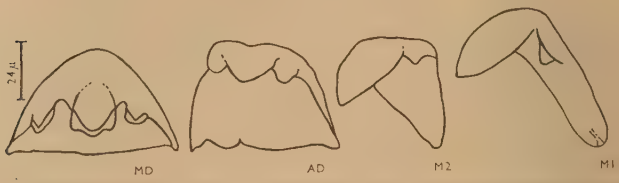

FIG. 2.—*Cypraea carneola*. Half-row of radular teeth. Teeth separated so that they do not overlap. AD, admedian ; M 1, outer marginal ; M 2, inner marginal ; MD, median.

of *Cypraea* (Kay, 1960), the animals of both species are consistent as regards these characteristics in the Hawaiian Islands. In *C. leviathan* the background colour is grey-brown, mottled with lighter brown ; the papillae are yellow and palmate. The tentacles and proboscis are black ; the foot is tan. In *C. carneola* the background colour of the mantle is red-brown ; the papillae are a lighter shade and conical. The tentacles and proboscis are yellow ; the foot is dark brown.

HABITAT AND DISTRIBUTION

Both *C. leviathan* and *C. carneola* are associated with coral reefs, as are most species of *Cypraea*. *C. leviathan* is one of the most commonly occurring species of *Cypraea* in the Hawaiian Islands, occurring in crevices at the seaward edge of basalt beaches, under coral blocks and rubble in inshore waters, at the breaker line of the outer edge of the reefs, and in large coral heads at depths up to 35 feet. *C. carneola* is less frequently

collected in Hawaii than is *C. leviathan*, as is evidenced by the occurrence of two to three specimens of *C. carneola* compared with fifty to sixty specimens of *C. leviathan* in Hawaiian collections. Specimens of *C. carneola* are, however, abundant in fossil material from Pleistocene limestone on the island of Oahu and in sub-fossil material from coral reef dredgings on the islands of Oahu and Kauai. Living specimens of *C. carneola* have been collected in the same areas as *C. leviathan* in the inshore waters of the Waikiki reef on Oahu (Burgess, personal communication), and at depths of 30 feet in regions of barren, dead coral where *C. leviathan* is noticeably absent (Harrison, personal communication).

The Schilders (1938–1939) attribute the distribution of *C. carneola* to the Indo-Pacific, from the east coast of Africa through the Pacific Ocean to the Hawaiian Islands. The range of *C. leviathan* is limited by the Schilders (1938–1939) to " Eastern Polynesia ", and they list it from various island groups including the Line Islands. An extensive collection of specimens of molluscs from the Line Islands which was turned up as a result of a storm in 1957, includes numerous specimens of *C. carneola* but no specimen conforming to the Schilders' description of *C. leviathan*. Collections from the Line Islands in the Bernice P. Bishop Museum in Honolulu, Hawaii, also lack specimens of *C. leviathan*. Specimens intermediate in the size range which has been considered here as distinguishing *C. leviathan* from *C. carneola*, that is, from 45 to 55 mm. in length, and several specimens exceeding 55 mm. in length have been recorded from the Sulu Sea and from the southern Caroline Islands (Mortlock Islands). However, radulae of these specimens have not been available for examination, and until such time as they are available the distribution of *C. leviathan* should most probably be limited to eastern Polynesia, with the exception of the Line Islands.

Discussion

Despite the anatomical distinction between the species of *C. leviathan* and *C. carneola*, it is not possible to distinguish the species on the basis of shell characters alone. The Schilders (1937) distinguished *C. leviathan* from *C. carneola* principally on the basis of the enormous size of the former. When shell lengths are compared, a distinctly bimodal curve is formed, but there is also an area of overlap between the two peaks which indicates that size alone is not a distinguishing characteristic of the two species. The other shell features noted by the Schilders are also somewhat difficult to use in distinguishing the two species: specimens which fall into the length range below 45 mm. may have an emarginate anterior end, several specimens which exceed 55 mm. in shell length from the Hawaiian Islands have a purple ring on the dorsum which is one of the distinguishing features of *C. carneola* and, finally, the terms " wider " mouth and " weaker " marbled sides are difficult to evaluate.

Although it is not possible to distinguish the species solely on the basis of conchological characters, the differences in radular and mantle features and the differences in habitat noted in the Hawiian Islands indicate that the Schilders' distinction between the species is useful. In the absence of

anatomical characters, it is suggested that identification of the species be made on the basis of a combination of conchological features and distributional range. Arbitrarily designating a maximum of 45 mm. shell length as characteristic of *C. carneola*, this species may be further identified by its distribution throughout the Indo-Pacific. Utilizing a minimum shell length of 55 mm. as characteristic of *C. leviathan*, this species may be further identified by limiting its range to eastern Polynesia exclusive of the Line Islands. Those specimens which are intermediate in shell length, or which exceed 55 mm. in length from the Central Pacific should be tentatively identified as *C. carneola*.

Summary

While it is not possible to distinguish between *C. carneola* and *C. leviathan* on the basis of conchological features, radular and mantle characteristics indicate that two species should be considered. Arbitrarily designating a 45 mm. maximum shell length as characteristic of *C. carneola* and 55 mm. minimum shell length as characteristic of *C. leviathan*, *C. carneola* is distributed throughout the Indo-Pacific and *C. leviathan* within eastern Polynesia with the exception of the Line Islands.

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ADDENDUM : A specimen of the *carneola-leviathan* complex 63 mm. in length from the Sulu Sea has been examined since this paper was submitted for publication. The radula of the specimen is of the *carneola* type, which adds further confirmation to the suggestion that the range of *C. carneola* is that of the Indo-Pacific, whereas that of *C. leviathan* is limited to the eastern Pacific.

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UPTAKE OF RADIOISOTOPES AND THEIR TRANSFER THROUGH FOOD CHAINS BY MARINE ORGANISMS

Annual Report, 1959–1960

By
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BIOLOGY AND MEDICINE

Hawaii Marine Laboratory

Annual Report
1959-1960

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Contribution No. 152
Hawaii Marine Laboratory

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ABSTRACT

The results of experiments performed to elucidate the mechanism by which the cichlid Tilapia mossambica maintains its osmotic equilibrium are reported. Using the previously designed compartmentalized tank, measurements have been made to show the ability of these fish to absorb strontium and sodium ions directly from the environment. All routes of absorption and excretion have been studied under variable experimental conditions. Strontium-85 has been used to measure Ca^{++} and Sr^{++} discrimination by fish. Analysis of the data shows that more of these cations pass through sea water adapted fish than through fresh water adapted fish; although in the former case a smaller quantity of radioactivity is measured. The difference between the quantity of these ions excreted in the urine could not account for the apparent discrimination in the fish. Experiments using Na-22 to study sodium activity show that rapid exchange of sodium occurs in both sea water and fresh water adapted fish. Because of the results obtained in these experiments, preliminary tests have been performed to show what effect stress and adrenocortical hormones may have on the selective absorption and excretion of ions. The entire body surface of Tilapia is permeable, and it is presumed that this permeability may be altered under stress and/or hormonal activity. No clear stress relationship has been obtained as yet; however, it appears that DOCA increases the permeability of the entire surface of the fish to Na-22, resulting in an increased accumulation and output of approximately twice that in the control fish.

Results of two artificial food web experiments are presented. Using zinc-65 to label sea water, results of experiments on the radiozinc accumulation by Chlamydomonas sp?, Artemia salina, and Chaetodon miliaris directly from solution and through feeding are presented. It is postulated that direct

absorption from sea water will be the most important method for accumulation of this element by these organisms. Because planktonic and benthic organisms appear to concentrate trace ions in the marine ecosystem, initial studies have been employed to show what effect chemical changes in the environment may have on the accumulation of isotopes by representative organisms. Data obtained on Chlamydomonas sp?, Artemia salina, and the brachiopod Lingula reevi, exposed to sea water containing Na-22 are presented. It is found that the sodium in these organisms reaches an equilibrium with the environment very rapidly. Indications point to the fact that the quantity of Na-22 accumulated is related to the ability of the organisms to regulate and selectively absorb ions.

Data on the relationship of organ size to fish size are presented. Comments on the handling and use of Tilapia mossambica for laboratory procedures are also included.

PROGRESS REPORT: ION TRANSFER THROUGH EPITHELIA OF FRESH AND SEA WATER ADAPTED TILAPIA MOSSAMBICA: COMPARTMENTALIZED TANK STUDY

Introduction

The long term in vivo study of the hydro-mineral metabolism in teleost fish started in 1957 has been continued. The experimental methods used to separate the surfaces of ion exchange and routes of ion excretion have been fully described in previous reports (1956-57, 1957-58, 1958-59). The surfaces of ion exchange of a fish are separated by securing the animal in the rubber dam which separates a small plastic tank into two compartments. The dam encircles the fish immediately in front of the pectoral fins exposing the head and gills to a compartment separate from that which contains the posterior part of the body. The fish is cannulated at both the papilla and anus so that urine and feces can be kept from the water of the back compartment and collected separately in rubber balloons. The pharyngeal end of the esophagus is ligated in some of the fish tested so that it is possible to assess the role of the alimentary tract as a route of ion absorption.

Three types of tests using animals adapted to each salinity were made with each ion studied: (1) fish with open esophagus exposed to the isotopic tracer in the front compartment water, (2) fish with ligated esophagus exposed to the isotopic tracer in the front compartment water, and (3) fish exposed to the isotopic tracer in the rear compartment water.

During the past year a more detailed study comparing calcium and strontium was completed and is reported below in Tables 1 to 9. A study of the metabolism of osmotically important sodium using Na^{22} as a tracer is now under way. Preliminary experiments to determine the amount of isotope, duration, etc. are completed and are reported in Table 10. As a corollary to, and parallel with

the sodium study, a study of the possible effect of hormones on the control of sodium movement through teleost fish is being initiated and results obtained thus far are reported here.

I. STRONTIUM STUDIED WITH STRONTIUM-85

Tables 1 to 7 list the accumulation of strontium-85 by T. mossambica when separate epithelia of the fish were exposed to both fresh and sea water enriched with Sr^{85}. Activity of a variety of parameters is recorded in micromicrocuries (μμc). The following notations apply to Tables 1 to 8: "Total through Fish" means the total amount of Sr^{85} that must have gone through the integument exposed to the compartment containing the Sr^{85} dose. "Ca^{++} + Sr^{++}" indicates the amount of calcium and strontium ions that were taken up through the integument exposed to the radioactive water in each particular case. This was calculated from the measured activity. "$\bar{X} \pm s$" is the mean ± the standard deviation of the mean for each set. "Gut" includes the gastrointestinal tract from esophagus to rectum. It should be noted here that we are assuming no discrimination between Ca^{++} and Sr^{++} with Sr^{85} acting as a tracer for both ions. Thus these values are a minimum, for if there is any discrimination, the true value would be higher. "Water" represents the total activity appearing in the compartment opposite the one containing the Sr^{85} dose and is activity which had passed through the fish. One can also conclude that the values for "Ca^{++} + Sr^{++}" are a minimum because some of the radioactivity must have gone back into the compartment from which it was picked up. It can be seen that more of these cations pass through sea water adapted fish than through those exposed to fresh water (c.f. Tables 1 and 2, etc.).

Table 1. Accumulation of Sr^{85} by fresh water adapted Tilapia mossambica in 9 hours when the isotope is added to the front compartment. Radioactivity is recorded in micromicrocuries (μμc).

	LIGATED			OPEN		
Live Wt. (gms.)	83.25	74.50	98.99	82.11	101.37	92.00
Dose/ml.	17.426	10.704	17.761	23.470	20.136	18.048
Blood (per ml.)	6.666	8.706	10.227	4.642	10.531	8.825
Total in Fish	409.749	302.942	507.932	308.083	721.109	392.257
Urine (per ml.)	.813	.186	1.391	.868	.092	.637
Urine (total)	2.439	1.008	2.073	5.170	.318	3.172
Feces	.762	-	-	-	.724	-
Gut	1.246	.351	.687	3.956	10.078	9.241
Water	42.998	37.789	47.568	47.236	32.100	40.372
Total thru Fish	463.860	350.896	568.487	365.131	764.783	444.626
$Ca^{++} + Sr^{++}$ (mg.)	.184	.226	.364	.107	.262	.169
	$\bar{X} \pm s = .258 \pm .071$ mg.			$\bar{X} \pm s = .179 \pm .055$ mg.		

Table 2. Accumulation of Sr^{85} by sea water adapted Tilapia mossambica in 9 hours when the isotope is added to the front compartment. Radioactivity is recorded in micromicrocuries (μμc).

	LIGATED			OPEN		
Live Wt. (gms.)	64.12	96.70	97.90	109.70	87.20	90.81
Dose/ml.	13.889	23.679	17.397	17.486	17.163	18.003
Blood (per ml.)	.280	.281	.297	-	.314	.359
Total in Fish	21.092	35.942	31.237	33.035	19.621	30.147
Urine (per ml.)	.246	.303	.286	.392	.244	.395
Urine (total)	.924	.909	.578	.684	trace	1.382
Feces	-	-	.032	1.241	.015	.940
Gut	.114	1.400	.671	26.869	25.600	17.881
Water	23.550	26.177	17.472	20.061	17.260	18.003
Total thru Fish	46.517	64.709	50.287	55.413	37.454	51.226
$Sr^{++} + Ca^{++}$ (mg.)	1.366	1.115	1.179	1.291	.890	1.161
	$\bar{X} \pm s = 1.220 \pm .110$ mg.			$\bar{X} \pm s = 1.114 \pm .167$ mg.		

Table 3. Accumulation of Sr^{85} by fresh water adapted Tilapia mossambica in 22 hours when the isotope is added to the front compartment. Radioactivity is recorded in micromicrocuries (μμc).

	LIGATED				OPEN			
Live Wt. (gms.)	114.25	138.20	107.80	123.71	121.81	111.87	120.31	115.21
Dose/ml.	11.732	13.777	13.290	11.484	14.451	15.413	13.648	11.571
Blood (per ml.)	8.135	11.509	5.101	12.323	7.744	11.143	14.232	10.621
Total in Fish	731.452	682.862	676.797	869.974	684.787	1343.634	1117.188	642.459
Urine (per ml.)	1.815	.833	-	1.389	1.531	2.866	3.528	1.893
Urine (total)	14.260	14.988	4.133	21.540	10.509	21.171	25.958	25.384
Feces	1.058	2.073	.256	.774	1.126	2.902	1.279	2.293
Gut	.696	.397	1.545	.131	13.304	14.843	9.903	10.710
Water	24.777	25.261	19.112	42.907	73.596	28.463	54.747	29.334
Total thru Fish	780.378	737.100	706.944	947.649	776.636	1404.411	1212.125	707.798
$Sr^{++} + Ca^{++}$ (mg.)	.459	.442	.367	.569	.372	.628	.613	.422
	$\bar{X} \pm s = .459 \pm .055$ mg.				$\bar{X} \pm s = .509 \pm .055$ mg.			

Table 4. Accumulation of Sr^{85} by sea water adapted Tilapia mossambica in 22 hours when the isotope is added to the front compartment. Radioactivity is recorded in micromicrocuries (μμc).

	LIGATED				OPEN				
Live Wt. (gms.)	84.81	102.01	100.04	96.38	83.39	74.70	99.01	97.20	102.71
Dose/ml.	14.473	12.981	12.492	12.931	12.604	15.629	13.052	12.879	12.391
Blood (per ml.)	.451	.515	.561	.454	.472	.858	.438	.407	.563
Total in Fish	48.923	57.665	43.016	41.664	39.082	60.885	43.468	31.141	47.739
Urine (per ml.)	.368	.497	.397	.214	.531	.593	.397	.505	.497
Urine (total)	.368	.637	.180	.200	.310	.117	.272	.311	.288
Feces	.038	-	.079	-	9.795	2.103	7.870	12.911	8.045
Gut	.156	.319	.416	.279	162.361	166.635	119.698	125.662	143.144
Water	26.177	24.284	21.721	15.237	19.985	23.255	16.964	18.236	22.926
Total thru Fish	79.978	83.098	65.894	57.555	59.849	85.115	61.142	50.095	71.516
$Ca^{++} + Sr^{++}$ (mg.)	2.255	2.612	2.152	1.816	1.937	2.224	1.911	1.587	2.355
	$\bar{X} \pm s = 2.209 \pm .281$ mg.				$\bar{X} \pm s = 2.003 \pm .266$ mg.				

Table 5. Accumulation of Sr^{85} by fresh water adapted Tilapia mossambica in 22 hours when the isotope is added to the back compartment. Radioactivity is recorded in micromicrocuries (μμc).

Live Wt. (gms.)	145.00	120.00	131.1	127.00	155.80
Dose/ml.	11.709	9.573	9.656	11.774	12.820
Blood (per ml.)	.513	.399	.544	.760	1.373
Total in Fish	204.860	263.766	285.792	389.681	539.784
Urine (per ml.)	-	.066	.242	-	-
Urine (total)	-	.297	-	-	-
Gut	.858	1.198	.774	1.158	2.374
Water	57.532	49.356	85.079	92.735	86.900
Total thru Fish	263.763	315.016	372.431	491.174	630.431
$Sr^{++} + Ca^{++}$(mg.)	.155	.227	.266	.289	.339
	$\bar{X} \pm s = .255 \pm .063$ mg.				

Table 6. Accumulation of Sr^{85} by sea water adapted Tilapia mossambica in 22 hours when the isotope is added to the back compartment. Radioactivity is recorded in micromicrocuries (μμc).

Live Wt. (gms.)	96.24	84.20	96.35	90.31	87.21	92.71
Dose/ml.	12.242	8.190	11.961	8.667	12.551	14.566
Blood (per ml.)	.299	.203	.233	.256	.169	.485
Total in Fish	52.421	26.291	50.391	28.304	23.811	28.206
Urine (per ml.)	-	.191	.247	.186	.150	.444
Urine (total)	-	.107	.291	.179	.086	.222
Feces	-	-	-	.027	-	.009
Gut	.426	.372	.535	.310	.668	.421
Water	14.534	2.006	6.842	12.259	13.793	17.145
Total thru Fish	67.680	28.979	58.292	41.334	38.527	46.488
$Ca^{++} + Sr^{++}$ (mg.)	1.742	1.444	1.988	1.946	1.253	1.302
	$\bar{X} \pm s = 1.613 \pm .297$ mg.					

Table 7. Accumulation of Sr^{85} by sea water adapted Tilapia mossambica in 9 hours when the isotope is added to the back compartment. Radioactivity is recorded in micromicrocuries (μμc).

Live Wt. (gms.)	94.67	100.08	89.20
Dose/ml.	14.243	17.900	18.210
Blood (per ml.)	.286	.241	.227
Total in Fish	21.994	17.911	16.206
Urine (per ml.)	-	.239	.202
Feces	-	-	-
Gut	.425	.394	.472
Water	13.626	20.893	14.530
Total thru Fish	36.328	39.558	31.435
$Ca^{++} + Sr^{++}$ (mg.)	1.040	.825	.704
	$\bar{X} \pm s = .856 \pm .141$ mg.		

Table 8. Accumulation of Sr^{85} by fresh water adapted Tilapia mossambica in 22 hours when Ca^{++} and Sr^{++} were added to the water in amounts equivalent to that contained in sea water. Radioactivity is recorded in micromicrocuries (μμc).

	FRONT				REAR	
	OPEN		LIGATED			
Live Wt. (gms.)	89.33	81.32	89.80	91.2		
Dose/ml.	13.580	15.854	14.513	16.519	12.274	10.900
Blood (per ml.)	.607	.588	.392	.693	.206	.250
Total in Fish	40.151	44.916	33.294	51.801	31.918	29.173
Urine (per ml.)	.415	.363	-	-	-	-
Urine (total)	4.207	2.954	-	-	-	-
Feces	-	trace	-	-	-	-
Gut	.590	.441	.228	.324	.366	.437
Water	9.721	9.387	6.419	10.715	7.017	8.917
Total thru Fish	55.276	58.286	40.333	63.533	39.507	38.777
$Sr^{++} + Ca^{++}$ (mg.)	1.623	1.500	1.134	1.569	1.312	1.451
	$\bar{X} \pm s = 1.432 \pm .161$ mg.					

It will be noted that the uptake of Sr^{85} in these fish is approximately the same as that for comparable sea water adapted fish living in sea water and not equivalent to comparable fresh water fish living in fresh water (c.f. Tables 3 and 4 and 5 and 6). This indicates that there is no salinity regulation of Sr (Ca) uptake. Rather the amount of these ions contained in the water determines their rate of exchange by the fish.

II. COMPARATIVE UPTAKE OF STRONTIUM AND CALCIUM

It is well known that calcium and strontium behave in a qualitatively similar manner in the animal body but there is a quantitative difference in the metabolism of the two cations. Several investigators have done comparative studies using mixed tracer doses in the same animal to attempt to determine where in the animal the discrimination occurs (7,17,fish;15,rat). In the 1958-59 annual report (pp. 21-26) we reported an experiment using Ca^{45} and Sr^{90} to measure the comparative accumulation of Sr^{++} and Ca^{++} by T. mossambica. Table 9 reports a similar study using the previously described compartmentalized tank and methods in an attempt to locate the sites of discrimination. It must be emphasized here that the entire amount of these ions excreted in the urine, determined from the radioactivity in the volume of urine excreted, could not account for the discrimination found.

Table 9. Comparative uptake of strontium and calcium in fresh water adapted Tilapia mossambica.

Portion Exposed	Total	Total	Total	Front	Rear	Rear
Duration	5 hrs.	2 days	3 days	22 hrs	5 hrs.	5 hrs.
	Strontium/Calcium					
Environmental Water	0.901	0.554	0.913	0.471	0.970	0.491
Blood	0.493	0.559	0.548	0.374	0.831	1.310
	Observed Ratio*					
Urine	0.937	0.924	-	0.799	0.811	1.015
Bile	0.509	0.515	0.696	2.355	0.411	0.300
Gut	1.085	0.610	0.711	0.480	0.669	0.172
Gill Rakers	0.469	0.478	0.472	0.694	0.716	0.302
Scales	0.447	0.621	0.460	0.557	-	0.212
Muscle	0.503	0.682	0.430	0.583	0.589	0.295
Bone	0.371	0.551	0.404	0.478	0.649	0.160
Feces	-	0.608	-	-	-	-

* $O.R. = \frac{Sr/Ca \text{ of sample}}{Sr/Ca \text{ of blood}}$ (Comar, C. L., R. Scott Russell, R. H. Wasserman, 1957, Science 126, pp. 1-8).

III. SODIUM

Sodium-22 was chosen as the tracer for the study of sodium metabolism because it is available in carrier free form, it has a long half life (2.6 years) and is a gamma emitter making sample preparation easier and less subject to technical errors. All samples were counted in a sodium iodide (Tl) well-type scintillation counter and single-channel analyzer with a 10 volt window setting. The discriminator on the analyzer was set to give the most efficient counting statistics as indicated by the ratio of sample counts squared over background counts (13).

The results of the preliminary experiments are recorded in Table 10. They indicate that sodium moves rapidly into the fish from the environmental water and that experiments of four hours duration would yield the greatest amount of information.

IV. STRESS DUE TO RESTRICTION OF FISH IN A DAM

The type of experimental technique used in this study has been justifiably criticized, because the "stress" conditions introduced by restricting a fish in the dam of the compartmentalized tank could upset its hydro-mineral exchange pattern " . . . . one hesitates to accept the results so obtained as representative of its normal physiology" (8). Therefore, an experiment was designed to assess the effect of this stress on the sodium uptake in fish. Tilapia paired as to weight, sex and age were chosen. One of the pair was secured in the dam of the compartmentalized tank. Large holes had been cut in the separating dam and the aerating stone placed so that the water was caused to circulate freely between the two compartments. The other fish of the pair was put into a similar but uncompartmentalized tank where it had room to move about freely. Three liters of tap water was put into each tank. The tap water in both tanks was

Table 10. Accumulation of Sodium-22 by fresh water adapted Tilapia mossambica. Activity recorded in micromicrocuries (μμc).

Region exposed to Na^{22}	Rear	Front	Front	Rear	Front	Front	Total
Duration	2 hrs.	2 hrs.	2 hrs.	4 hrs.	4 hrs.	4 hrs.	24 hrs.
Fresh Wt. (gms.)	139.9	114.0	96.2	115.5	124.1	118.7	103.4
Eyes	.01	19.51	2.77	.45	25.84	17.74	52.24
Spleen	.02	6.18	.15	.06	.69	.79	.63
Gonads	.10	20.93	.05	.10	34.66	29.31	7.89
Heart	.04	1.77	.12	.07	1.25	1.85	2.06
Head (incl. skin)		256.87		8.35	627.71	321.19	631.34
Kidney	.21	3.50	.12	.04	4.66	5.42	5.63
Gall Bladder/Bile	.09	7.13	.11	.07	6.13	8.92	48.94
Liver/Pancreas	.14	7.97	.17	.48	11.88	10.36	7.65
Gut*	.54	20.80	2.09	1.06	37.86	37.14	62.14
Gills		100.96		9.22		140.36	123.59
Skin (rear)		16.05		.72	37.14	26.40	31.50
Scales		42.55		2.08	57.38	60.08	60.72
Blood (per ml.)	.19	19.29	.59	.36	28.12	62.93	35.66
Urine (per ml.)			.02	.01	4.05	.83	3.46
Miscellaneous**	35.23	242.89	-	13.68	262.18	409.93	591.76
Water (per ml.)***	.01	.02	-	Bkgd.	.02	.02	3.83
Water (total)***	5.67	32.00	-	Bkgd.	.50	.32	-
Dose (per ml.)	2.27	13.58	.65	12.59	12.54	13.60	3.87

* Includes gastrointestinal tract from esophagus to rectum.

** Includes rest of tissues either not listed above, or for which no values are listed, e.g., in first column 35.23 represents value for urinary bladder, etc. as well as head, gills, etc.

*** Activity in compartment opposite dose compartment.

replaced after one hour by three liters of tap water enriched with Na^{22}. After 4 hours exposure the fish were removed from the radioactive water, rinsed for one minute in running tap water, bled and pithed. The fish were prepared for radioassay as previously described. The results are shown in table 11.

Although the data accumulated to date on this problem do not clearly indicate the true stress effects of the compartmentalized tank, future experiments will be designed to gain as much information as possible on this problem.

Table 11. Comparative four-hour accumulation of sodium-22 by fresh water adapted Tilapia mossambica under stress. Activity recorded in counts per minute.

Fresh Wt.(gms.)	Dose	Treatment	Total in Fish	Total in Viscera
106.5**	6,106	Stressed	1,213,866	62,287
109.5*	-	"	-	-
92.7	228	"	108,888	
81.6	177	"	30,288	
99.1**	6,375	Control	649,707	19,785
102.4**	1,765	"	255,169	26,806
91.3	279	"	4,285	
89.2	205	"	32,571	1,921

* Fish died before completion of four-hour interval.

** Fish counted with 10 volt window (compared to 1 volt with rest of fish).

PROGRESS REPORT: THE EFFECT OF ADRENOCORTICAL HORMONES ON THE PERMEABLE SURFACES OF TELEOST FISH

Although present evidence (12, 14, 16) demonstrates that adrenocortical and posterior pituitary hormones do exert regulatory control over the hydro-mineral metabolism of teleosts, there is no conclusive evidence in the literature to indicate which target organs are affected. The present study was designed to attempt to determine the regulatory effect on the probable target organs.

Sexton found a depression of sodium uptake with desoxycorticosterone acetate (DOCA) but no effect on sodium loss in goldfish. On the other hand, Holmes found an increased output of sodium after trout were given a sodium load. Although Nandi and Bern found cortisone (E) in both fresh water and sea water adapted fish, Sexton measured no change in sodium uptake when this hormone was given to goldfish.

The compartmentalized tank technique forms the nucleus of our experimental approach to the question: The ion permeability of what tissues or organs are under hormonal control?

For each hormone considered, a simple preliminary experiment was performed to determine whether the accumulation of sodium ion is altered by administration of the hormone.

Groups of paired fish were injected intraperitoneally. One group was injected with the hormone and the other group was injected with a similar volume of saline isotonic to the fish's blood. Five milligrams hydrocortisone acetate, eight milligrams desoxycorticosterone acetate (DOCA) were injected per fish 24 hours prior to immersion in Na-22 enriched fresh water. Compartmentalized tank fish were given three injections of DOCA of 1.66 mg. each prior to the experiments. Controls were injected with similar volumes of isotonic saline.

The fish were then placed in aquaria containing sodium-22 enriched water for a suitable time interval. Using this procedure, if there is a significant difference in the uptake of sodium-22 with a given hormone, the hormone will then be used in more detailed studies in the compartmentalized tank.

Table 12 records the results of preliminary experiments with desoxycorticosterone acetate (DOCA) and hydrocortisone acetate. Results with the latter are inconclusive, and more individuals must be tested before it can be determined whether sodium uptake by Tilapia mossambica is influenced by hydrocortisone.

The compartmentalized tank experiments completed to date using desoxycorticosterone acetate are presented in Table 13.

Although inconclusive, and more data is available which has not been analyzed, it appears that DOCA increases the permeability of the entire surface of the fish to sodium-22. Therefore (Table 13) in both front and rear compartments there is an increase in sodium accumulation and output approximately twice that in the control fish.

More data is needed to determine the threshold for hydrocortisone and DOCA activity in the fish before conclusive statements of permeability can be made.

Table 12. Effect of hydrocortisone and DOCA on sodium metabolism of fresh water adapted Tilapia mossambica. Activity recorded in micromicrocuries (μμc).

Fresh Wt. (gms.)	Dose	Treatment	Duration	Total in Fish	Total in Viscera
58.0	.109	hydro-	22¼ hrs.	19.440	1.252
51.5	"	cortisone*	"	25.442	1.432
83.5	"	"	"	17.941	1.175
53.0	.116	Control	"	25.266	1.242
51.5	"	"	"	26.797	1.948
79.0	"	"	"	28.457	2.395
25.6	3.467	DOCA**	25 hrs.	403.176	12.086
19.3	"	"	"	876.359	43.698
12.5	"	"	"	426.898	17.819
29.0	3.229	Control	"	398.682	13.257
16.6	"	"	"	549.812	20.368
13.4	"	"	"	260.010	14.628

* 5 mg. hydrocortisone acetate ("Hydrocortone") injected intraperitoneally per fish.

** 8 mg. desoxycorticosterone acetate (DOCA) injected intraperitoneally per fish.

Table 13. Effect of desoxycorticosterone acetate (DOCA) on sodium metabolism of fresh water adapted Tilapia mossambica held in the compartmentalized tank for four hours. Activity recorded in micromicrocuries (μμc).

Fish Wt. (gms.)	Treatment	Compartment with Dose	Total in Fish	Total in Water**	Total through Fish
117	DOCA*	Front	1775	90	1865
119	"	Rear	62	11	74
124	Control	Front	1114	50	1168
119	"	Front	1095	32	1128
116	"	Rear	36	Bkgd.	37

* Each fish given three intraperitoneal injections of 1.66 mg. each of DOCA.

** Total Na^{22} appearing in the compartment without the dose.

RADIOISOTOPE TRANSFER VIA ARTIFICIAL MARINE FOOD WEBS

I. Some aspects of the ecological transfer of Zn-65 in an artificial marine ecosystem

Introduction:

Continued research by the Beaufort group (4, 5, 6), and the Hanford, Washington group (1, 11) has only served to emphasize that the accumulation of zinc by marine organisms is highly variable. Despite the mass of data available, no attempt has yet been made to show the importance of chronic exposure of aquatic organisms to Zn-65 in the media. The most satisfactory data at the present time indicates that the accumulation of zinc is very rapid in marine animals. It has been shown, for example, that some phytoplankton, zooplankton and fish may accumulate zinc in amounts many thousand times that of the medium while other algae may exclude it. No such studies have been made to show what effect transfer through the food webs may play in increasing or decreasing concentrations already present from solution.

The ecologist is interested in the ability of organisms to transfer materials at each trophic level in the ecosystem. The radioecologist is interested in the methods of accumulation and which method will be most important in effecting maximum and minimum concentrations at each level.

The purpose of this experiment was to measure the accumulation of Zn-65 directly from the environment by three marine organisms. Feeding as a method of accumulation was tested to discover whether the rate of absorption by this means was equivalent to direct absorption from the water.

Methods:

The experiments were divided into three parts: 1) Accumulation of Zn-65 by unicellular algae, Chlamydomonas sp?, 2) Accumulation of Zn-65 by Artemia salina, and 3) Accumulation of Zn-65 by Chaetodon miliaris.

In each instance the organisms were cultivated in media containing varying concentrations of Zn-65 for varying time intervals.

Samples of each organism were removed at given intervals to determine the amount of radioactivity accumulated. All samples were counted using a NaI (Tl) well scintillator with a pulse height analyzer.

Samples of algae were removed and cell counts made. The samples of algae were centrifuged at 3500 CPM for 25 minutes, the supernatant decanted, the cells washed three times in "cold" sea water and then counted for radioactivity.

The brine shrimp, Artemia salina, were cultured in large flasks containing Zn-65 enriched sea water. For radioassaying, samples were removed, blotted, rinsed three times in "cold" sea water and counted in the well-scintillation counter.

The reef fish Chaetodon miliaris was chosen because it is a common tropical marine fish occurring in shallow water, is omnivorous, easy to care for in the laboratory and readily available. Hence it is an ideal animal for these experiments. The fish were maintained in running sea water aquaria (five per 20 liter container) until they became acclimated and would feed readily. They were then transferred to 100 liters of filtered non-circulating sea water (20 per 100 liters) and Zn-65 added in appropriate concentrations. At suitable time-intervals five fish were removed, weighed, rinsed in fresh water, viscera removed and weighed, and all placed in crucibles for dry ashing. Weighed aliquots of ash were placed in vials and assayed for radioactivity.

Results:

The results of growing Chlamydomonas in sterile sea water containing Zn-65 at varying concentrations but without added nutrients are given in Table 14. Five concentrations of Zn-65 were used (281 - 350,000 cpm). Daily assays of Zn-65 activity were made for all culture concentrations but data for only one

Table 14. Rate of accumulation of Zn^{65} by Chlamydomonas sp? from sea water containing varying isotope concentrations.

Time (days)	Total CPM Sea water	Total CPM Cells	cpm/cell	Total No.Cells
2	2800	$8.1x10^2$	$2.8x10^{-5}$	$2.9x10^7$
	2800	$9.7x10^2$	$4.6x10^{-5}$	$2.1x10^7$
3	2800	$15.1x10^2$	$2.4x10^{-5}$	$6.3x10^7$
	2800	$9.1x10^2$	$1.4x10^{-5}$	$6.5x10^7$
4	2800	$16.6x10^2$	$2.2x10^{-5}$	$7.6x10^7$
	2800	$18.1x10^2$	$1.9x10^{-5}$	$9.6x10^7$
7	281	$2.6x10^2$	$1.5x10^{-6}$	$16.6x10^7$
	281	$2.5x10^2$	$1.9x10^{-6}$	$13.0x10^7$
	2800	$22.9x10^2$	$2.6x10^{-5}$	$8.8x10^7$
	2800	$22.5x10^2$	$2.1x10^{-5}$	$10.7x10^7$
	3400	$26.0x10^2$	$1.8x10^{-5}$	$14.6x10^7$
	3400	$26.0x10^2$	$2.0x10^{-5}$	$13.1x10^7$
	30500	$240x10^2$	$1.9x10^{-4}$	$12.4x10^7$
	30500	$230x10^2$	$1.6x10^{-4}$	$14.6x10^7$
	350000	$350x10^2$	$4.3x10^{-4}$	$8.2x10^7$
	350000	$350x10^2$	$4.5x10^{-4}$	$7.7x10^7$
9	281	$2.5x10^2$	$2.6x10^{-6}$	$9.4x10^7$
	281	$2.6x10^2$	$2.1x10^{-6}$	$12.1x10^7$
	2800	$20.3x10^2$	$2.6x10^{-5}$	$7.8x10^7$
	2800	$18.8x10^2$	$2.6x10^{-5}$	$7.2x10^7$
	2800	$22.1x10^2$	$2.8x10^{-5}$	$7.8x10^7$
	2800	$21.4x10^2$	$2.6x10^{-5}$	$8.2x10^7$
	2800	$21.9x10^2$	$2.1x10^{-5}$	$10.4x10^7$
	2800	$25.9x10^2$	$2.4x10^{-5}$	$10.8x10^7$
	2800	$21.4x10^2$	$3.6x10^{-5}$	$6.0x10^7$
	2800	$20.4x10^2$	$2.5x10^{-5}$	$8.2x10^7$
	3400	$26.0x10^2$	$2.4x10^{-5}$	$10.9x10^7$
	3400	$27.0x10^2$	$2.5x10^{-5}$	$10.6x10^7$
	30500	$200x10^2$	$2.0x10^{-4}$	$10.0x10^7$
	30500	$150x10^2$	$1.4x10^{-4}$	$10.6x10^7$
	350000	$450x10^2$	$5.5x10^{-4}$	$8.2x10^7$
	350000	$420x10^2$	$4.5x10^{-4}$	$9.4x10^7$

isotope concentration in the first seven days are presented in the table; because in all cases the amount accumulated in less than seven days was a function of concentration of inoculum. Two outstanding features of the cultures are apparent in the table. First, a higher level of Zn-65 activity per cell occurs in the first two days after inoculation, but by three days it has reached its optimum concentration. Note particularly that the cpm/cell at 4 to 7 days is approximately the same, except at exceptionally high concentrations of the medium. The second feature to be noted is that optimum growing conditions for the cells under constant illumination are reached in 7 days. Thus the total number of cells, the total counts and counts per cell reach optimum conditions at the same time. By nine days they begin to decrease as available nutrients are used and cultures become contaminated.

The accumulation of Zn-65 by Artemia salina directly from sea water is given in Table 15. Only two concentrations of Zn-65 in sea water were used, and it can be seen that the amount accumulated was highly variable; and at a very low level, even at 24 hours (1440 minutes). Nevertheless, it can be seen that by 60 minutes these animals had accumulated all that was possible directly from the water.

The results of the experiments using Chaetodon miliaris are presented in Table 16. Because of difficulties found in maintaining large numbers disease-free in non-circulating water and changing the water without changing its concentration, these experiments were performed at only two isotope concentrations. More data are available for the 660x103 cpm/liter than the lower concentration, because the fish lived more satisfactorily in the aquaria with the higher concentration. It was found that the animals showed a progressive increase in activity which was a function of the weight of the animals. By twenty-one days (504 hours) they had accumulated the maximum quantity of Zn-65 (exchange equi-

Table 15. Rate of accumulation of Zn-65 by Artemia salina L. from sea water containing varying isotope concentrations.

Time (min.)	No. & Sex	Total Activity (cpm)	CPM/Artemia	Water Activity cpm/cc
5	10 ♀	51	5.1	1450
	10 ♂	111	11.1	1450
	10 ♀	63	6.3	4000
	10 ♂	30	3.0	4000
10	10 ♀	44	4.4	1450
	10 ♂	151	15.1	1450
	10 ♀	268	26.8	4000
	10 ♂	44	4.4	4000
30	10 ♀	27	2.7	1450
	10 ♂	193	19.3	1450
	10 ♀	767	76.7	4000
	10 ♂	149	14.9	4000
60	10 ♀	13	1.3	1450
	10 ♂	111	11.1	1450
	10 ♀	1206	120.6	4000
	10 ♂	174	17.4	4000
120	10 ♀	17	1.7	1450
	10 ♂	145	14.5	1450
	10 ♀	945	94.5	4000
	10 ♂	482	48.2	4000
1440	10 ♂	451	45.1	4000

Table 16. Rate of accumulation of Zn-65 by Chaetodon miliaris from sea water at varying isotope concentrations.

Time (hrs)	Total Fish Wt. (gm)	Fish Wt. minus GI Tract (gm)	Wt. GI Tract (gm)	Total Activity (cpm)	Activity minus GI Tract (cpm)	Activity GI Tract (cpm)	Water Activity cpm/liter x 10^3
19	34	32	2	1312	749	563	165
	44	41	3	2640	1929	711	
	33	31	2	1273	902	371	
	19	18	1	2325	2199	126	
	38	35	3	2593	2116	477	
	14	13	1	3473	2989	484	
	42	39	3	1833	1156	677	
24	27	25	2	7171	2329	4842	660
	37	34	3	6950	1655	5295	
	31	29	2	7724	3902	3822	
	27	25	2	12251	3091	9160	
	36	34	2	5520	2241	3279	
27	32	30	2	1245	555	690	165
43	25	23	2	1743	821	922	165
48	33	31	2	1832	850	982	165
	22	20	2	8545	2795	5750	660
51	38	35	3	2326	978	1348	165
72	23	21	2	6454	1318	5136	165
	39	36	3	16585	5979	10606	660
	29	27	2	9494	4253	5241	
	50	45	5	16966	7102	9864	
	47	42	5	29295	7051	22244	
	38	35	3	11589	5091	6498	
91	45	40	5	6009	2823	3186	165
168	41	38	3	15073	6926	8147	165
	16	15	1	8001	2368	5633	
	34	32	2	56037	29497	26540	660
	51	47	4	54580	21263	33317	
	40	36	4	59278	24978	34300	
	44	41	3	75192	32260	42932	
	28	25	3	63975	12796	51179	

Table 16. (continued)

Time (hrs)	Total Fish Wt. (gm)	Fish Wt. minus GI Tract (gm)	Wt. GI Tract (gm)	Total Activity (cpm)	Activity minus GI Tract (cpm)	Activity GI Tract (cpm)	Water Activity cpm/liter x 10^3
264	48	44	4	92310	48117	44193	660
	15	13	2	87583	15083	72500	
	24	22	2	48042	19962	28080	
	21	19	2	38696	17114	21582	
	9	8	1	25583	13506	12077	
504	47	43	4	136250	71131	65119	660
	49	44	5	155425	88430	66995	
	44	40	4	160559	81117	79482	
609	37	33	4	222055	103558	118497	660
	45	39	6	185444	80115	105329	
	39	35	4	164583	105345	59238	

librium not growth). This is found by examining the column "Activity minus GI Tract." It has been shown elsewhere (17) that the amount found in the GI Tract may be highly variable depending on a number of factors.

Discussion:

To properly assess the role of any radioisotope as a contaminant in any given ecosystem a number of criteria must be satisfied which cannot always be satisfied in practical applications, but which in experimental procedures can be controlled. Of prime importance in ion metabolism is the fact that all members of any given ecosystem be cultured under identical conditions. Then, there must be no loss due to leaching of ions when transfer to new media is made, also growth must not occur during the interval of the experiment, and finally when using radioactive isotopes the organism must not be adversely affected by the presence of the stable isotope in the medium. These criteria are often difficult to reconcile in any given experiment.

Chipman (4, 5, 6) states that he finds a marked accumulation of zinc-65 by brine shrimp and higher concentrations in females than in males. Furthermore, he points out that he finds a higher concentration in the brine shrimp when zinc has been available in the food than when it was present in the water alone. Our experiments do not confirm this; in fact, rather the opposite seems to obtain. If one calculates concentration ratios, as does Chipman, our values are lower for Artemia. It does not seem justifiable to calculate concentration ratios in food web studies, but specific activities would be more meaningful. To properly assess the importance of radiocontaminants it is necessary to determine which method (ingestion or exchange) will contribute the most to the concentration found in any given organism.

Using the present data we can arrive at an approximation of the numbers of organisms necessary at each trophic level to equal that absorbed from the water

alone. Assuming a sea water concentration of $4\text{-}8 \times 10^6$ cpm Zn-65/liter, at equilibrium each organism should show the following concentrations due to direct absorption of the radioactive zinc from solution:

Chlamydomonas sp?	5.0×10^{-4} cpm
Artemia salina	150 cpm
Chaetodon miliaris	2×10^5 cpm

Hypothetical calculations from these values show that if all the zinc-65 obtained through feeding were absorbed and retained; each brine shrimp would require 3×10^5 cells and each Chaetodon would require 1300 brine shrimp to obtain the same activity as that from the water alone.

Our data show that sterile sea water without added nutrients will maintain a colony of Chlamydomonas of 3×10^6 cells per cc. Thus, it is conceivable that 0.1 cc of cells would produce the required number of counts equivalent to that from the water alone. However, preliminary experiments show that a maximum of 5 per cent of the activity contained in Chlamydomonas is retained by Artemia fed on the algae labelled with zinc-65. Therefore, at least 2 cc of labelled algae must be filtered by each Artemia to reach a concentration equivalent to the water alone. This is not inconceivable.

More serious problems are encountered, however, when one considers the transfer from brine shrimp to fish. If no loss of labelled zinc occurs, then it would require 1300 brine shrimp to obtain the concentrations found in the fish. Preliminary experiments show that a maximum of 10 per cent is retained when Chaetodons are fed zinc-65 labelled brine shrimp. Consequently, 13,000 Artemia would be required to produce the desired zinc-65 level in each fish, and calculations based on this value show that 3.9×10^9 labelled Chlamydomonas would be required to achieve the equilibrium concentration in Chaetodon. This is equivalent to labelled Chlamydomonas in 1.3 liters.

Experiments using Sr-85 have shown that a 100 gram marine fish swallows approximately 15 cc of sea water in 24 hours. Assuming 20 grams as the average weight of a Chaetodon, it would swallow approximately 3 cc of water in 24 hours and thus about 400 days to swallow 1300 cc. This indicates that direct absorption from sea water will be the more effective route for accumulation of zinc-65 by marine fish.

It should be pointed out that the values arrived at here are conservative estimates and probably should be higher. However, this does not imply that there are no important organisms which concentrate ions through food chains. The great mass of data accumulated in field observations by other laboratories only serve to emphasize this fact. The variability from species to species and habitat to habitat show quite clearly that ion metabolism in aquatic environments is a much more complex problem than that of simple diffusion or exchange through food webs.

II. A study of the ability of various marine organisms to maintain their sodium concentration using Na-22 as a tracer.

Introduction:

The distribution of organisms in the marine environment shows good correlation with the ability of the organisms to maintain their osmotic pressure and salt balance. In animals this has been accomplished in several ways, e.g., impermeable exoskeleton, selective absorption of ions, and selective excretion by means of kidney structures. The littoral fauna of a given marine habitat perhaps has the greatest abundance of regulating organisms because of the necessity to withstand wide fluctuations in the physical and chemical environment. Because of the abundance of food close to shore, the littoral fauna supplies some of the most commercially important food organisms. The possibility of selective absorption and excretion of ions by these animals therefore, may present serious problems with respect to disposal of radioactive wastes from any source.

Numerous assays have been made on the accumulation of certain dissolved ions by the littoral marine benthic fauna (4, 5, 6, 11). As yet no attempt has been made to correlate the observed concentrations in these organisms with changes in the chemical and physical environment, nor comparisons made with pelagic or sublittoral relatives.

This work was initiated to show the effects of different ionic concentrations in sea water on the ability of several marine species to accumulate radioactive Na-22 dissolved in sea water. Because sodium is so variable in littoral benthic habitats, it was believed it would be a suitable ion to investigate possible changes in ion absorption and excretion by these species

Methods:

The procedures used in these experiments are the same as those employed in

the previously reported experiments using zinc-65. However, the isotope used in these experiments was Na-22;and the brachiopod, Lingula reevi Donaldson was used as a test organism. These are conspicuous animals on reef flats which may be exposed to expectional daily fluctuations in temperature and salinity. The ability of these animals to maintain their osmotic equilibrium and salt concentrations make them ideal organisms for studying the effects of changing environmental ionic concentrations and isotope accumulation.

The present studies have been concerned only with normal sea water.

Results:

The results of preliminary experiments using Chlamydomonas grown in sea water labelled with Na-22 (Table 17) show that they very rapidly accumulate Na-22. By one day the cells have accumulated the optimum quantity, indicating a rapid equilibrium time.

Exposure of Artemia salina in sea water labelled with Na-22 also shows a very rapid accumulation of sodium. Preliminary experiments using only male Artemia had shown that exposure of one hour was sufficient for the animals to reach equilibrium with their environment. The results of the experiment reported in Table 18 are from Artemia exposed to sea water containing three concentrations of Na-22 and with all potassium replaced by sodium or all sodium replaced by potassium. Although the experiments have not been carried to the equilibrium time, the table shows a faster initial influx of Na-22 when there is no sodium in the medium. However, the greatest accumulation occurs in those animals which were exposed to sea water without sodium.

The results of the experiments on Na-22 influx and efflux in the brachiopod Lingula reevi are presented in Table 19. As in the other two experiments it can be seen that Na-22 accumulated by Lingula occurs at a very rapid rate. The table indicates that by 10 to 20 minutes after exposure to sea water labelled with Na-22

the animals have come into equilibrium with their environment. This is evidenced by the fact that after this time there is no significant increase in Na-22 activity in the animals, even with prolonged exposure. The rate of efflux is at a much slower rate. By two hours only 10 - 20 per cent of the accumulated Na-22 had been excreted.

Discussion:

The experiments performed at this time are too preliminary to warrant detailed discussion, but several things must be noted for comparison with other published data. With all organisms used thus far the indication is that sodium is much more rapidly exchanged with the environment than the other ions which we have studied. Thus by one day (and even minutes) the Na-22 concentration in organisms may come into equilibrium with that of the culture medium.

Chipman (4, 5) has pointed out that Cs-137 is rapidly accumulated from sea water by a number of marine organisms (i.e. Artemia Tigriopus, Fundulus, etc.). His data show a rapid equilibrium time similar to that which we have found for sodium. However, the data indicate that Cs-137 is accumulated in much larger quantities than Na-22. This difference may be due to at least two factors. 1) The specific activity of any sea water containing Na-22 is always very low, because of the large amount of carrier sodium present, while the specific activity of Cs-137 will be always very high. 2) The effect of competition by other ions may also play an important role. It is known that potassium will be preferentially accumulated by cells to the exclusion of sodium, and there is some indication that cesium may replace potassium. These two considerations were the basis for the experiments using brine shrimp in "sodium-free" and "potassium-free" sea water. Because these animals can live for long periods at extreme concentrations they are ideally suited for such experiments. The present data show that, to a slight extent, more sodium may be exchanged by these animals

when potassium is absent from the environment.

The ability of Lingula to live under extreme environmental conditions and their anatomy also make them admirably suited for such experiments. Being supplied with a shell, it is conceivable that under adverse conditions they may close the valves and be impermeable to changing salt concentrations. However, they are attached to the substrate by an elongated, fleshy peduncle which is richly supplied with blood. This peduncle cannot be withdrawn, and it indicates that these animals are exposed to the environment at all times. The Lingula data further indicate that sodium is much more rapidly accumulated by these animals and in larger quantities than has been found in other animals. As yet no studies of changes in ion concentration have been performed, but they should prove very rewarding for comparative purposes. These animals have been given little physiological attention, and these processes may prove as unique as their fossil history.

Table 17. Rate of accumulation of Na-22 from sea water by Chlamydomonas sp?

Time (days)	Total No. Cells (5cc)	Cells Total Activity (CPM/5cc)	CPM/cell	Sea Water Total Activity (CPM/5cc)
1	7.9×10^5	24	3.0×10^{-5}	125
	2.5×10^5	23	9.0×10^{-5}	48
	2.5×10^5	33	13.0×10^{-5}	110
	15.5×10^5	25	2.0×10^{-5}	50
	5.0×10^5	22	4.0×10^{-5}	55
2	9.6×10^5	14	1.5×10^{-5}	50
	9.6×10^5	19	1.9×10^{-5}	47
	9.6×10^5	17	1.7×10^{-5}	40
	9.6×10^5	19	1.9×10^{-5}	44
6	15.2×10^5	17	1.1×10^{-5}	33
	15.2×10^5	19	1.2×10^{-5}	52
	15.0×10^5	18	1.2×10^{-5}	37
	15.0×10^5	18	1.2×10^{-5}	53

Table 18. Rate of accumulation of Na-22 from sea water and artificial sea water by Artemia salina L.

Time	No. Artemia & Sex	Total Activity (CPM)	CPM/Artemia	Sea Water Activity (CPM/cc)	
1 min.	5 (3 ♀ , 2 ♂)	2	0.4	370)	
	5 (3 ♀ , 2 ♂)	-	-	4000)	no Na^+
	5 (3 ♀ , 2 ♂)	15	1.5	8000)	
	5 (3 ♀ , 2 ♂)	-	-	370)	
	5 (3 ♀ , 2 ♂)	4	0.8	4000)	no K^+
	5 (3 ♀ , 2 ♂)	2	0.4	8000)	
5 min.	5 (3 ♀ , 2 ♂)	-	-	370)	
	5 (3 ♀ , 2 ♂)	5	1.0	4000)	no Na^+
	5 (3 ♀ , 2 ♂)	3	0.6	8000)	
	5 (3 ♀ , 2 ♂)	1	0.2	370)	
	5 (3 ♀ , 2 ♂)	6	1.2	4000)	no K^+
	5 (3 ♀ , 2 ♂)	7	1.4	8000)	
1 hr.	5 (3 ♀ , 2 ♂)	1	0.2	370)	
(60 min.)	5 (3 ♀ , 2 ♂)	7	1.4	4000)	no Na^+
	5 (3 ♀ , 2 ♂)	24	4.8	8000)	
	5 (3 ♀ , 2 ♂)	2	0.4	370)	
	5 (3 ♀ , 2 ♂)	15	3.0	4000)	no K^+
	5 (3 ♀ , 2 ♂)	25	5.0	8000)	
3 hrs.	10 (♂)	6	0.6	4000)	Sea
19 hrs.	10 (♂)	9	0.9	4000)	Water

Table 19. Rate of accumulation and loss of Na-22 by Lingula reevi Donaldson from sea water with isotope concentration of 1500 cpm/cc.

Time (min.)	Inflow Total Activity (CPM)	Outflow Total Activity (CPM)
10	125	118
20	104	-
	129	-
30	116	-
	134	96
40	128	-
	137	-
	126	103
60	125	-
	125	-
	138	103
80	118	-
	132	-
	132	92
100	120	100
	147	-
	122	86
120	120	88
	110	74
	118	84
	129	90

COMMENTS ON TILAPIA MOSSAMBICA AS A GOOD EXPERIMENTAL ANIMAL FOR OSMOREGULATION STUDIES

I. Introduction

The cichlid, Tilapia mossambica, used in these experiments is gaining in popularity with research workers as an experimental animal, particularly for ion transport studies. It is hardy and can be kept indefinitely and bred successfully in laboratory tanks. It has been used as an experimental animal in the Hawaii Marine Laboratory since 1954.

Because so few experimental methods with fish in general and T. mossambica in particular are available in the literature, it seems advisable to include observations we have made of this fish and a description of the techniques that we have developed for handling it.

II. Experimental Handling

Tilapia tolerate well the confinement necessary in a laboratory but they become stressed when they are chased with a net or handled excessively or improperly. Such "trauma" is indicated by color change, rigid erection of the dorsal fin and resistance by the fish. We have learned that the fish relaxes and trauma can be kept to a minimum if it is held so that the dorsal fin is flattened against the body and the eyes are kept covered. In addition, the animal will remain relaxed indefinitely if it is held upside down. We have adopted this posture and use it uniformly throughout all our experimental work.

III. Salinity Tolerance

Tilapia mossambica are completely euryhaline, easily adapted to either fresh or sea water. Three generations of tilapia have been spawned and reared successfully in sea water in our laboratory tanks although the species is

normally reared in fresh water. There is no apparent correlation between breeding and the ability of the fish to adapt to various environmental salinities.

IV. Experimental Bleeding

Previous work (3) from this laboratory has confirmed Forster's (9) observation that it is easy to withdraw unclotted blood from a fish if its gills are kept immersed. There is undoubtedly a dehydration of the blood of some species of fish as a result of asphyxiation (10). All experimental procedures should be performed without removing the fish from water.

To remove blood from the heart, a size #24 hypodermic needle is used. On the ventral side of the fish between the pectoral and pelvic girdles there is a soft spot where the heartbeat can be felt. The hypodermic needle is placed in the center of this area and slowly advanced into the fish's cardinal cavity following a line passing approximately 3/4 of a centimeter posterior to the eye. The apex of the ventricle of the heart will be touched. This is confirmed by the feel of the heartbeat through the lightly held syringe. When the needle is advanced another 1/4 of a centimeter, the heart is entered. This is verified by the appearance of blood in the syringe when slight negative pressure is applied to the plunger.

Blood also can be withdrawn from the kidney sinus. A size #18 hypodermic needle is placed under the scales in line with the horizontal septum, as indicated by the ridge on the scales, and directly below the fifth dorsal spine. By advancing the needle gently cephalad, it enters easily between the lateral muscles, past the rib, above the air sac and into the renal sinus until the spinal column is reached. Then the needle is withdrawn slightly. Blood must be extracted as rapidly as possible. Care should be taken never to remove

the operculum from water. When properly withdrawn, tilapia blood does not clot.

V. Relationship between Organ and/or Tissue Weight and Total Body Weight

Many of our experiments with tilapia during the past several years have required complete and careful dissections of the fish. Data showing the relationship between total body weight and tissues and/or organ weights for both fresh water and sea water adapted tilapia are presented in Tables 20 and 21. Statistical analysis of the first 19 fish indicate that there is a significantly greater amount of bile contained in the gall bladder of fish adapted to sea water ($t = 4.20$, $t_{.01} = 2.90$). With all the other organs and tissues, the relationship of organ to total body weight is the same regardless of the salinity of the external environment.

Table 20. Relationship between organ and/or tissue weight and total body weight of fresh water adapted Tilapia mossambica, expressed as per cent of body weight.

Organ and/or tissue	Range	$\bar{X} \pm s$*	N*
Eyes	0.74 to 2.04	1.47 ± 0.28	22
Heart	0.05 to 0.12	0.07 ± 0.01	22
Stomach/esophagus	0.10 to 0.74	0.34 ± 0.14	22
Gall Bladder/bile	0.18 to 0.98	0.45 ± 0.21	20
Spleen	0.03 to 0.08	0.05 ± 0.01	21
Liver/pancreas	0.26 to 2.25	1.03 ± 0.51	21
Intestines	0.99 to 4.04	2.26 ± 0.95	22
Gonads	0.07 to 2.77	0.59 ± 0.74	22
Urinary Bladder	0.01 to 0.19	0.04 ± 0.05	13
Kidney	0.06 to 0.65	0.27 ± 0.14	22
Gills	4.31 to 7.12	5.22 ± 0.72	13
Brain/spinal cord**	0.12 to 0.70	0.35 ± 0.15	13
Skin**	5.17 to 12.31	9.25 ± 3.26	12
Scales	2.43 to 6.29	4.32 ± 0.95	11
Muscle**	35.17 to 47.32	41.28 ± 3.56	11
Bone**	6.52 to 13.17	10.87 ± 1.69	11

* "$\bar{X} \pm s$" = arithmetic mean ± standard deviation of the mean.
"N" = number of items in the sample.

** Values based on cooked tissue.

Table 21. Relationship between organ and/or tissue weight and total body weight of sea water adapted Tilapia mossambica; expressed as per cent of body weight.

Organ and/or Tissue	Range	$\bar{X} \pm s$*	N*
Eyes	1.59 to 2.46	1.93 ± 0.24	16
Heart	0.06 to 0.24	0.10 ± 0.05	16
Stomach/esophagus	0.17 to 0.58	0.36 ± 0.13	15
Gall Bladder/Bile	0.40 to 0.87	0.61 ± 0.14	13
Spleen	0.02 to 0.08	0.05 ± 0.01	16
Liver/Pancreas	0.17 to 1.26	0.80 ± 0.28	16
Intestines	0.79 to 5.59	2.85 ± 1.17	16
Gonads	0.05 to 2.94	0.48 ± 0.73	16
Urinary Bladder	0.02 to 0.19	0.07 ± 0.04	14
Kidney	0.19 to 0.53	0.34 ± 0.10	16
Gills	4.65 to 6.24	5.31 ± 0.55	16
Brain/Spinal cord**	0.23 to 0.64	0.44 ± 0.14	15
Skin**	6.29 to 14.84	11.01 ± 2.25	14
Scales	2.70 to 5.43	4.07 ± 0.72	15
Muscle**	31.65 to 73.97	44.92 ± 9.18	14
Bone**	7.29 to 15.01	12.18 ± 2.12	14

* "$\bar{X} \pm s$"=arithmetic mean ± standard deviation of the mean.
"N" = number of items in the sample.

** Values based on cooked tissue.

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PROFESSIONAL STAFF AND ACTIVITIES

S. J. Townsley - Assistant Marine Biologist and Assistant Professor of Zoology
Employed half-time.

Student training:

Susumu Kato - Graduate assistant employed half-time on A.E.C. Contract No. AT(04-3)-56, September 1959 - May 1960.

Hester Kobayashi - Student trainee under N.S.F. undergraduate research training program, summer 1959.

Consultant for A.E.C. Project AT(04-3)-235.

Paper presented at A.I.B.S. meetings, Penn State University, 1959.
"The effect of environmental calcium and strontium on the accumulation of Ca-45 and Sr-90 by Tilapia mossambica."

Della F. Reid - Junior Marine Biologist. Employed full time.

Paper presented at A.I.B.S. meetings, Penn State University, 1959.
"Ion exchange through epithelia of fresh and sea water adapted teleost studied with radioactive isotopes."

Winifred T. Ego - Assistant in Marine Biology. Employed one-fourth time.

Mrs. Ego left employment in May, 1959 to care for her newly adopted infant. In January, 1960 she returned to work one-fourth time.

Monthly rain samples were collected and sent to the New York Operations Office of the U. S. Atomic Energy Commission.

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ADDITIONS TO THE CHECK LIST OF HAWAIIAN BIRDS

By Miklos D. F. Udvardy[1]

The latest revision of the Hawaiian check-list appeared in 1958 by E. H. Bryan, Jr. (Check-list and summary of Hawaiian birds. Honolulu. pp.28), a revision of his earlier check-lists.

During 1958-59, while in Honolulu, I have worked on the distribution and migration of continental shore and water birds of the Hawaiian Islands. Mr. Bryan generously allowed me to use his files of notes, and I also checked through the published literature. The additional faunistic data thus collected, together with several recent records, are listed below as an addition to Mr. Bryan's 1958 check-list.

Seventeen species of the list that follows are new on the check-list. Data on ten species are included, because their rare occurrence is not completely documented in the previous check-list. In the case of the rest the status of the bird has to be changed, as a consequence of additional records, from chance visitor to occasional visitor or migrant, or from this to regular visitor.

The bulk of additional faunistic data come from the years since the early 1940's. They coincide in time with the start of extensive popular interest in Hawaiian birds, and in many cases also with the activity of several trained observers. Thus the increase of the check-list and the changing status of migrants in many cases reflect the well-known phenomenon, i.e. that 'not the birds are rare, but the bird watchers.'

Very few of these data are documented with museum specimen or otherwise acceptable evidence. However, sight records by reliable observers were accepted for the previous check-lists. In this present list such published sight records are included, provided the publication did not leave doubt to the identity of the sighted bird.

New species of the Hawaiian check list, i.e. those not included in previous check-lists, are indicated by an "N" in the last column of the list.

April 1, 1961

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
2	Diomedes immutabilis Rothschild Laysan Albatross	Oahu, 1947	17	NW Hawn Isl, rarely Main Isl.
2A	Fulmarus glacialis (Linnaeus) Fulmar	Oahu, 1959	51	N. Chance visitor.
3	Puffinus pacificus chlororhynchus Lesson Wedge-tailed Shearwater	Niihau, Maui	74	NW and Main Hawn Isl.

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Contribution No. 153 of the Hawaii Marine Laboratory.

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
3A	Puffinus griseus (Gmelin) Sooty Shearwater	Midway,1957	45	N. Chance visitor.
5	Puffinus newelli Henshaw Newell's Shearwater	Kauai,1894-1907	74	Breeds on Niihau, Kauai, Oahu, Molokai, Maui, Hawaii.
		1956	76	
		Oahu,1938	13	
		1954	73	
		1959	39	
		Hawaii,1894-1907	74	
8	Bulweria bulwerii (Jardine & Selby) Bulwer's Petrel	Off Maui and Hawaii	74 2	NW and Main Hawn Isl.
18A	Bubulcus ibis Linnaeus Cattle Egret	Kauai,Oahu,Maui, Molokai,Hawaii,1959	6	N. Introduced from Florida, 1959.
18B	Casmerodius albus (Linnaeus) Common Egret	Oahu,1944	12	N. Chance visitor.
28	Branta canadensis (Linnaeus) Canada Goose	Oahu,1949	66	Occasional on Main Hawn Isl.
		1958-59	52,54,89	
		Molokai,1936	57	
		Maui, 1936	57	
		1941	55	
		1959	53,54	
		1960	54,58	
		Hawaii,1958	54	
27	Branta nigricans (Lawrence) Black Brant	Oahu, 1945	29	--
		1960	58	
		Molokai,1939	56	
26	Philacte canagica (Sewastianov) Emperor Goose	Oahu,1960	41	--
24	Chen. h. hyperborea (Pallas) Lesser Snow Goose	Oahu,1958-59	25,89	--
35	Anas carolinensis Gmelin Green-winged Teal	Oahu,1940	61	Occasional migrant.
		1953,54	54	
		1958	89	
		Maui,1941	55	
		1956,60	54	
		Hawaii,1954	54	
34	Anas discors Linnaeus Blue-winged Teal	Kauai,1946	65	Chance visitor.
		Oahu,1946	30	
		Maui,1960	54	
34A	Mareca penelope (Linnaeus) European Widgeon	Oahu,1944	12	N. Chance visitor.
		1957	79	
		Hawaii,1952	54	

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
37	Mareca americana (Gmelin) American Widgeon	Midway, 1956-57	45	Regular visitor in small numbers from North America.
		Oahu, 1940	61	
		1944	11,12	
		1950,53,54	54	
		1954	71	
		1956	36,54	
		1957	79	
		1958,59	16,27,38,54,81,89	
		Maui	7	
		1951,54,57	54	
		Molokai, 1951,59	54	
		Hawaii, 1952-54	54	
		1956-60	54	
38A	Aythya americana (Eyton) Redhead	Midway, 1956	45	N. Chance visitor.
38B	Aythya collaris (Donovan) Ring-necked Duck	Maui, 1948-49	83	N. Chance visitor.
38C	Aythya valisineria (Wilson) Canvasback	Maui, 1948-49	83	Chance visitor.
		1960	54	
38D	Aythya marila (Linnaeus) Greater Scaup	Oahu, 1946	64	N. Chance visitor.
		Hawaii, 1902	43	
41A	Aythya affinis (Eyton) Lesser Scaup	Oahu, 1946	64	Chance visitor.
41B	Aythya fuligula (Linnaeus) Tufted Duck	Midway, 1959	18	N. Chance visitor.
39	Bucephala albeola (Linnaeus) Bufflehead	Laysan, 1897,1912	1	Occasional visitor.
		Oahu, 1929,38	59	
		1943	9	
		1953	33	
		1956	46	
		Maui, 1959	54	
		Hawaii, 1959	54	
39A	Clangula hyemalis (Linnaeus) Oldsquaw	Midway, 1958	8	N. Chance visitor.
39B	Oxyura jamaicensis (Gmelin) Ruddy Duck	Oahu, 1945	67	N. Chance visitor.
		Hawaii, 1952	54	
44	Pandion haliaetus (Linnaeus) Osprey	Oahu, 1939,40	63	Occasional straggler.
		1956	47	
		1960	80	
		Hawaii, 1951	68	
		30°N.Lat-140°W.Long, 1957	84	
83	Charadrius semipalmatus Bonaparte Semipalmated Plover	Oahu, 1957	82	--
		1958	25	

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
83B	Charadrius alexandrinus Linnaeus Snowy Plover	Oahu, 1958a 1958b	25 49	N. Chance visitor.
83A	Charadrius vociferus Linnaeus Killdeer	Oahu, 1952	31	Chance visitor.
81	Squatarola squatarola (Linnaeus) Black-bellied Plover	Midway, 1940,41 Oahu, 1939 1953 4x 1954 4x 1955 4x 1956 4x 1957 3x 1958 2x 1959 2x 1960 1x Maui, 1959 2x Hawaii, 1900	14 60 4,20,32,87 5,21,22,34 23,69,70,77 24,35 78,79,82 16,49 26,75 80 10 42	Regular migrant in small numbers.
88	Capella gallinago delicata (Ord) Common Snipe (Wilson's Snipe)	Molokai, 1902 Hawaii	56 56	--
88A	Numenius phaeopus variegatus (Scopoli) Whimbrel	Midway, 1940 1957	14 45	N. Chance visitor.
86A	Totanus melanoleucus (Gmelin) Greater Yellowlegs	Midway, 1941 Oahu, 1958	14 89	Chance visitor.
86B	Totanus flavipes (Gmelin) Lesser Yellowlegs	Midway, 1941	14	Chance visitor.
90	Erolia acuminata (Horsfield) Sharp-tailed Sandpiper	Midway, 1907,54 Kauai, Molokai, Hawaii Oahu, 1960	1 7 44	--
91	Erolia melanotus (Vieillot) Pectoral Sandpiper	Midway, 1940,41 Oahu, 1892 1939 1941 Maui, 1951 Hawaii, 1900	14 7 60 62 3 42	Occasional visitor.
91A	Erolia alpina (Linnaeus) Dunlin	Midway, 1956 Laysan, 1913	45 1	N. Chance visitor.
91B	Limnodromus scolopaceus (Say) Long-billed Dowitcher	Oahu, 1944 1954 1958	12 21 81	Occasional visitor.
91C	Ereunetes mauri Cabanis Western Sandpiper	Oahu, 1958	25,89	N. Chance visitor.

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
94A	Stercorarius pomarinus (Temminck) Pomarine Jaeger	Oahu, 1952	19	Regular migrant in small numbers.
		1956	36	
		1957	79	
		1958	38	
		1959	27	
		1960	80	
101	Larus hyperboreus Gunnerus Glaucous Gull	Oahu, 1959	51	Occasional straggler.
		Hawaii, 1936	56	
100	Larus glausescens Naumann Glaucous-winged Gull	Midway, 1956	45	--
		French Frigate, 1954	72	
		Oahu, 1960	40	
98	Larus occidentalis Audubon Western Gull	French Frigate, 1953	72	Introduced, but not established. Chance arrival.
96.	Larus argentatus Pontoppidan Herring Gull	Oahu, 1957	48	Occasional straggler.
		1958	51	
		Hawaii, 1958	15	
97	Larus californicus Lawrence California Gull	Hawaii, 1958	54	--
95	Larus delawarensis Ord Ring-billed Gull	Oahu, 1944,45	12	--
		Hawaii, 1959	54	
103	Larus philadelphia (Ord) Bonaparte's Gull	Laysan, 1912	1	Occasional straggler.
		Oahu, 1952	19	
		1957	48	
		1960	40	
		Maui, 1957	54	
		Hawaii, 1959	54	
107A	Sterna albifrons Pallas Least Tern	Oahu, 1958	50	--
112	Columba livia Gmelin Rock Dove	Midway, 1956	45	Well established on Midway and Main Hawaiian Islands.
136A	Tyto alba pratincola (Bonaparte) Barn Owl	Hawaii, 1958-59	37,86	N. Introduced from California
137	Asio flammeus (Pontoppidan) Short-eared Owl	Midway, 1907,49	1	Native to all the Main Hawaiian Islands; in places numerous on grassy forehills. Occasional on Midway.
		1956,57	45	

No. in Check-list	Name	Locality and Date	Reference No.	Changed Status of the Species
221A	Lonchura malacca atricapilla Black-headed Mannikin	Oahu, 1959	88	N. Introduced as cage bird since 1936 from India. Breeding on Ewa, Oahu, 1958.
148	Mimus polyglottos (Linnaeus) Mockingbird	Maui, 1959 Hawaii, 1960	90 28	Established on Oahu and Maui. Seen on Hawaii.

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Acanthophora, a Possible Invader of the Marine Flora of Hawaii

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Acanthophora, a Possible Invader of the Marine Flora of Hawaii[1]

MAXWELL S. DOTY[2]

IN THE FALL OF 1952, a small algal fragment was brought to the author from Pearl Harbor[3] by Mr. Charles Cutress. In April, 1953, a much larger piece of the alga was brought in by Mr. Spencer Tinker, who had found it washed ashore near the Waikiki Beach Laboratory of the University of Hawaii. Both collections were readily determined as representing a species of *Acanthophora* Lamouroux (1813: 132), a rhodophytan genus. This genus is distinct and clearly recognizable among the floral elements occurring in this part of the world. A search of the literature and the herbaria available revealed no Pacific records of this genus from the Hawaiian Islands or, with one exception, east of the Western Caroline and Marianas islands north of the equator.

Other collections during succeeding years, and field observations as well, revealed a huge increase in the abundance of the species in Hawaii during the next few years. In May, 1953, Dr. D. W. Strasburg found this alga "in abundance" at Keehi Lagoon, between Pearl Harbor and the Port of Honolulu on the leeward side of Oahu. Later the same month, a dense growth of the alga was found by the author (numbered 10774) and Dr. E. Y. Dawson at Hauula, north of Honolulu, on the windward side of the island of Oahu. Parts of this collection are the earliest collections from Hawaii represented in both the Bishop Museum and the University of California herbaria. From that time on, *Acanthophora* has been so common on the leeward side of Oahu that it has not often been preserved as an herbarium specimen.

Finally, during June, 1956, Dr. Otto Degener collected and sent in a specimen (his no. 24105) from Mokuleia on the windward shore of the island of Oahu, northwestward from Honolulu, collected by himself, Miss Marie Neal, and Dr. Constance Hartt, with the annotation ". . . ubiquitous some distance within the reef; observed very rare here last year. This is first time aggressive marine alga threatening native kinds." Certainly it appears to be replacing (crowding out) elements of the native flora. Degener, who has paid close attention to the reef population at this particular site, feels the alga probably was not there until, at most, 2 years before this collection was made.

Not only have frequency of observance and density of standing crop increased, but the distribution has been that of a progressive encircling of Oahu, one of the few islands of the Hawaiian Group where *Acanthophora* has been found.

The alga has spread to Kauai, another of the Hawaiian Islands. Mr. Jan Newhouse has passed on local stories that the alga was not found around Kauai until about 1954 or 1955, and the observation that it is now ubiquitous. This genus was recorded from Kauai by Kohn and Helfrich (1957: 243). Their mention of the genus was based on observations of Newhouse about November, 1956, and was not included among the algae they collected and had identified by Dawson at the time their earlier work (Helfrich and Kohn, 1955) was completed in October and November of 1955. It has been found neither among our earlier extensive collections from Kauai nor by Newhouse among his, but during August 1960, Dr. Charles Lamoureux and Mr. Tadayuki Kato made a collection (Lamoureux coll. no. 1542) of *Acanthophora* on Kauai at Poipu Beach.

Specific, though in part cursory, searches during 1960 for *Acanthophora* on the islands of

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[3] According to Mr. Mikihiko Oguri, this algal collection probably came from West Loch, between Laulaunui Island and the northwestern shore.

Hawaii, Lanai, and Maui variously by Robert K. S. Lee, Mikihiko Oguri, Warren Wilson, and the author have resulted in no traces of this alga being found with one exception. The exception is a collection made by the author (numbered 19431), Mrs. Meng Sung Doty and Mr. Lee along the north shore of Lanai in November, 1960, where the alga was washed onto the muddy sand beach in abundance, free or attached to shells, coral, or even rocks up to 2 lb. in weight. Unfortunately, the Kawaihae area on Hawaii and the shores of Molokai have not been specifically searched for this genus, but our collections made during earlier years from these places do not include *Acanthophora.*

In the light of the ability and persistent vigor of the algologists who have at one time or another concerned themselves with the marine algae of the Hawaiian Islands, e.g., Drs. W. A. Setchell, Josephine Tilden, G. F. Papenfuss,[4] I. A. Abbott, and earlier, the Misses Minnie Reed and Marie Neal, and Mrs. Nina H. Loomis, it seems unlikely that this alga would have been overlooked had it been consistently present. It is a conspicuous alga. The older Polynesians in Hawaii seem to have had no name for *Acanthophora.* If pressed for a Polynesian name nowadays, the common man professing native acumen will apply local names such as manauea, the name widely used formerly for species of other genera, such as *Gracilaria* (now usually referred to by the Japanese name, ogo).

Identifying the Hawaiian alga has led to a consideration of the differences purported to exist between the several species reported in the Pacific. Many variants can be found in the material that has been available for this study from both the Atlantic and the Pacific, but for the present it is felt that the many forms found might best be treated as variants of one species. *Acanthophora spicifera*[5] (Vahl) Boergesen (1910) is the name for this species having priority insofar as we know.

Among the most common names[6] considered here as having been applied to the taxon *A. spicifera,* as found in the Pacific, is *A. orientalis* J. Agardh (1863). In describing *A. orientalis* as a new species, J. Agardh listed the Marianas Islands, of which Guam (13° N., 145° E.)[7] is one, as the source of one of the two collections he had seen. The other collection was probably from Manila Bay (14° N., 121° E.) in the Philippines. Safford (1905: 30–32) says that in the Marianas, the islands Guam, Rota (14° N., 145° E.) and Tinian (15° N., 146° E.) were visited by the Freycinet expedition. The material of this expedition from these islands is believed to be the source of one of the two collections Agardh reported. In his text, Safford (1905: 177 f.) lists *A. orientalis* from Guam and we presume this to be based on the Freycinet record, since Safford also says Dumont d'Urville collected several new species of algae on Guam. We ourselves have seen no specimens from Guam, despite a search through the several collections, now in our possession, which were made there by Mr. Ernani Meñez in 1960.

Except for the reports from Hawaii, the genus is not known to occur in the Pacific east of the Marianas other than in the Ponape region, where it has been reported by Yamada (1944: 44) as *A. muscoides* (L.) Bory from Ant (7° N., 158° E.), an atoll 8 mi. to the southwest of Ponape.

It is common about the large subcontinental or continent-related islands of the far western Pacific and, as *A. spicifera,* according to Womersley (1958), in northern Australia. The genus is reported (Kanda, 1944: 749) from Palau (7° N., 134° E.) as *A. orientalis.* As *A. thierii* Lamx. the genus is recorded from the Admiralty Islands (probably 2° S., 147° E.) and Tongatabu (21° S., 175° W.) by Dickie (1875*b*: 238, 235, resp.) and from Torres Straits (10° S., 143° E.) by Dickie (1876: 447). *Acanthophora* is common in the warmer part of the Atlantic, and Lamouroux (1813) believed the genus to be circumequatorial.

It seems entirely possible that this species

[4] In correspondence, Dr. Papenfuss tells us that neither he nor Setchell found this genus in Hawaii, and that the only Hawaiian specimens in the University of California herbarium are duplicates of the Hauula collections sent in by Dawson and mentioned above.

[5] Basonym=*Fucus spiciferus* Vahl, 1802.

[6] The only similar species not mentioned otherwise here appears to be *A. aoki* Okamura, 1934.

[7] The approximate latitude and longitude in degrees is given for the convenience of those interested in the location of the places named.

could have arrived in Hawaii from the west on a ship bottom, i.e., a man-made facilitation of the oceanic drift method of dispersal. This would be the carrying of an organism "upstream."[8] The progressive increase in abundance around the island over several years' time, and, recently, what appears to be a leveling off in abundance, is considered evidence of an introduction into the Honolulu–Pearl Harbor area. The Honolulu–Pearl Harbor shore area is the part of the state having the greatest traffic with regions of the world where *Acanthophora* has been known as a common component of the flora for a long time. This area is not climatically extreme for the state. However, since Honolulu has been in contact with the East and West via ship for centuries, it seems likely that some recent unusual occurrence may have implemented this transport. The three following events have come to our attention and are considered in this regard.

First, the recent warming of North Pacific waters (e.g., since the low temperature year of 1955 at Christmas Island, 2° N., 157° W.) would not, it seems, be accountable for the following reasons: first, the warm temperatures did not begin until after the alga was well known in Hawaii, and, secondly, there has been little abnormality of water temperatures in the Hawaiian area itself.

A second unsual event that may have led to the introduction is the increase in traffic between the Honolulu–Pearl Harbor area and the Far East during World War II, 1941–45, and during the Korean Police Action, 1950–53. The idea is that a number of small introductions at nearly the same time might have provided together a sufficiently large inoculation for the species to become established. From the rate of spread we tend to exclude the first period. The second is more timely. There is no special evidence that would lead one to choose this latter as the probable period, though such changes in oceanic traffic have been held to account for the distribution of barnacles in some cases elsewhere in the world.

A heavily fouled barge, the "Yon 146," towed to Pearl Harbor from Guam, provides an example of the third, and more specific, type of event that may have led to the establishment of *Acanthophora* in Hawaii. The idea here is that one heavily "fouled bottom" could have provided a sufficiently large inoculation for the species to become established. Upon arriving in Pearl Harbor February 3, 1950, this "fuel oil barge (non-self-propelled)" was placed in a dry dock. The Pearl Harbor dry docks are about 12 km. by water and 8 km. in a straight line from the place Cutress collected the first material of *Acanthophora* found in Hawaii. The dry dock is about 30 km. by water from the place Tinker first found this species of *Acanthophora.*

Fish and gastropod collections were made both from the growths, often 3–8 in. thick, on the barge and from the small pools left under the barge in the otherwise dry dry dock. They were made, at least in part, on April 10, 1950, and variously by Tinker, George Campbell, and Kenneth A. Wong. This vessel, 200 ft. long, 56 ft. in beam, concrete-hulled, under different descriptive names, has been mentioned as a possible means of fish introduction by several authors, e.g., by Gosline and Brock (1960: 26), who have studied collections made on it. The same vessel is reported by Edmondson (1951: 183, 212) as having brought in invertebrates which have become established,[9] such as the brachyuran crab, *Schizophrys aspere,* common to the far western Pacific. Chapman and Schultz

[8] This is the customarily postulated direction of migration in deriving the populations of Hawaii, as summarized by Zimmerman (1948), and Gosline and Brock (1960). Ladd (1960) has emphasized a somewhat different possible mode of origin for the populations of the Pacific islands.

[9] From accounts of long-time residents of the area, known distribution of the species, and information in the literature, it seems to me almost certain that *Cotylorhizoides pacificus* (Mayer) and *Cassiopea medusa* (Light), both Rhizostomae, were accidentally introduced to the Pearl Harbor area during the 1941–45 period. The type locality of these two medusae is the Philippine Islands. In Hawaii both were restricted to Pearl Harbor until about 1950. About this time, *Cassiopea* appeared in Honolulu Harbor and the Ala Wai Canal. Later, 1953–54, *Cotylorhizoides* appeared in Kaneohe Bay. Until the time of my departure (December, 1955) neither medusa was known from the other Hawaiian Islands, Line Islands, Marshalls, Gilberts, etc. Both forms undoubtedly came to Hawaii as scyphistoma on ships or the like, as neither are medusae of the open ocean.—C. E. Cutress, June, 1960.

(1952) concluded there was no evidence among the fish records they obtained that species had been brought to Hawaii in the fouling population on that barge. Dr. C. M. Burgess, who, along with Campbell and Tinker, provided much of the specific information on these events, told the author that the species of the molluscan genus *Cypraea* brought in on this vessel, the "Yon 146," did not become established in Hawaii, and this has been affirmed by Dr. Alison E. Kay, who is a student of this genus.

In commenting upon *Tripterygion hemimelas* Kner & Steindachner and *Ecsenius hawaiiensis* Chapman & Schultz, two blennioid fishes reported to have been collected from the pools of water in the dry dock holding the "Yon 146," Strasburg (1956: 245 f.) notes that the specimens of *T. hemimelas* were similar to a species from the Samoan Islands (14° S., 171° W.). The service record of this barge, as far as the author has been able to trace it, indicates that it was tied up at Apra (13° N., 145° E.), Guam, from 1945 to the date it was towed to Pearl Harbor, a period of about 4 years. Thus is does not seem likely that it would have been directly the means by which a Samoan fish would have been introduced into Hawaiian waters. Whether *T. hemimelas* occurs in Guam or not is not known to the present author.

Possibly the blennioid fish, *Omobranchus elongatus* (Peters), was brought to Hawaii (Strasburg, 1956: 257) from the Samoan area along with chunks of reef rock bearing living specimens of the giant clam, *Tridacna*. At least for the present, this splendid possible avenue of introduction is discounted; though *Acanthophora* has been reported (Reinbold, 1896) as *A. orientalis* from Upolu (14° S., 171° W.), Western Samoa.

Individual ships have been cited previously as the means by which algae have been introduced into the Central Pacific. Dickie (1875*a*: 33) published a note to the effect that *Ulva latissima* Linnaeus was introduced to Mangaia (22° S., 158° W.) in the Cook Islands when a whaling ship from the Antarctic was wrecked there on the reef in 1852. There is the possibility, however, that the wrecked ship merely provided a favorable habitat, in which habitat an ulvoid alga interpreted by Dickie as representative of this specific taxon appeared. In form the ulvoid algae, of the larger benthic algae, are among the most plastic in respect to environmental conditions.

Kohn (1959: 81) records *Acanthophora* (using the binomial *A. orientalis*) from Kaneohe Bay, Oahu, Hawaii, where it was the substratum upon which the eggs of *Conus quercinus* were found attached in February, 1956. *C. quercinus* has been recorded for Hawaii for many years (e.g., Bryan, 1915: 454), but the alga for only a few years. If *C. quercinus* is very host-specific in its egg-case depositing, this observation of Kohn's could be taken to imply long presence of *Acanthophora* in Hawaii. Though the alga is independent of the mollusc, if the mollusc is restricted to the algal species for egg-case deposition, the alga would probably have had to be here first and it would have taken many years for the mollusc to develop modified egg-case depositing habits including *Acanthophora* as a host. Our impression[10] is, however, that egg-case attachment by molluscs is not very specific, substratum-wise, and therefore, that there is no implication in Kohn's record that *Acanthophora* grew in the islands, say, in 1915.

After considering the types of events described above, we feel that it is most likely that *A. spicifera* arrived recently in Hawaii via the fouled bottom of a ship. Aside from the ordinary ship traffic, similar opportunities for introduction by vessels other than the "Yon 146" are known. One of these opportunities is provided in the case of a similar vessel, the "Yogn 41," which was towed from Subic Bay (15° N., 120° E.) in May, 1947. This "gasoline barge (non-self-propelled)" was drydocked in February, 1950, in Pearl Harbor. While Subic Bay is unknown phycologically, *A. spicifera* is common in the general area, e.g., in Manila Bay a few miles to the south it is abundant. However, from the timing of the events and from the rate of spread after the first specimens were found, it is believed that

[10] A letter received since from Prof. J. M. Ostergaard supports this impression concerning the specificity of egg-case deposition by *Conus* and tells us of his "finds" of *C. quercinus* in the Honolulu Harbor area as dead shells in 1902 and 1905 and as living shells in 1915.

an earlier vessel would be much less likely a source of the introduction of *A. spicifera* than the "Yon 146."[11]

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[11] Though we do not know what other biological consternation may be associated with this vessel, it may cause little more for Hawaiian biologists, for it is reported to have been sunk in Subic Bay on October 16, 1955.

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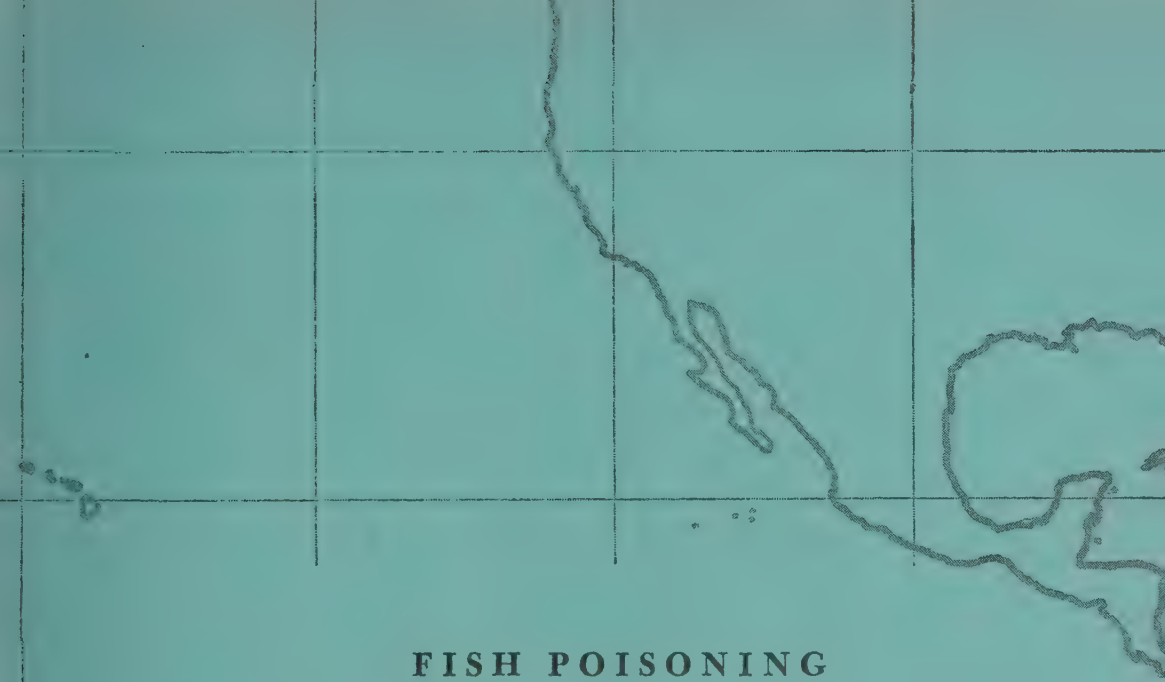

FISH POISONING

In the Tropical Pacific

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Fish Poisoning in the Tropical Pacific

INTRODUCTION

A number of reef fishes living in tropical seas have been reported poisonous to eat and their ingestion has caused serious intoxications in humans. This condition exists around many tropical Pacific islands, resulting in illness and occasional deaths among the inhabitants, and often profoundly affecting their welfare by depriving them of a major source of food. A recent spread of fish poisoning in the Pacific, and a concern over the possible relationship of radioactive contamination to the problem, coupled with increased demands on the fishery resources in the Pacific area, have been instrumental in attracting the attention of research workers to the problem in the past few years. Despite the extensive literature which documents the existence of fish poisoning, its prevalence in the tropics, and the seriousness of the problem in some areas, there appears to be a paucity of basic information published and considerable confusion on the subject.

The term ichthyosarcotoxism is often used in medical literature to describe a specific type of intoxication caused by the ingestion of the *flesh* of poisonous fishes. Fish poisoning encountered in the tropical Pacific may be due to the ingestion of portions of the fish other than the flesh and therefore this term will not be used in this discussion.

PRESENT AND PLANNED RESEARCH

The Hawaii Marine Laboratory of the University of Hawaii, in cooperation with a number of national agencies of the United States government, has instituted a broad program of research on fish poisoning in the Pacific. This research emphasizes a study of the chemistry, pharmacology, and biological origin of the toxin involved in ciguatera fish poisoning. A study of epidemiological factors involved in the magnitude, spread, geographic distribution, and the host of clinical syndromes produced by various types of fish poisoning is also being pursued.

The need for a comprehensive epidemiological study of fish poisoning in the Pacific was recognized when an attempt was made to determine the relationship between laboratory studies of various categories of toxic fish and the clinical picture of the illness in humans resulting from the ingestion of these fishes. Sporadic reports of fish poisoning have included numerous and varied symptoms, but accurate descriptions of outbreaks that might reveal information on the type of poisoning, site and mode of action of the toxin, and associated environmental phenomena affecting the toxicity of the fish is often incomplete or absent. A recent limited epidemiological survey of fish poisoning in the Southern Marshall Islands revealed the type of information on the magnitude and seriousness of the problem that can be gained from a planned and coordinated undertaking. As a result of recommendations made following this survey, a more comprehensive epidemiological survey of fish poisoning in the entire tropical Pacific is being undertaken by scientists at the Hawaii Marine Laboratory, the South Pacific Commission, and the National Institutes of Health. This study will be closely coordinated with laboratory and field studies on

the biology, chemistry, and pharmacology of various types of fish poisoning in an effort to obtain information that will enable medical and administrative personnel on Pacific islands to better recognize and control the fish poisoning problem in their area.

Fish poisoning occurs at widely scattered and remote islands over a tremendous expanse of the Pacific Ocean. Practically speaking, the only feasible means of conducting such a survey is through a joint cooperative venture with the medical personnel of the area. The success of this survey, therefore, depends upon the wholehearted cooperation of all qualified personnel who might have contact with, and are interested in, the problem of fish poisoning. This illustrated publication containing the latest information that is available on the various recognized categories of fish poisoning is being distributed to medical personnel in the tropical Pacific. It is hoped that this material will assist in the recognition, diagnosis, and perhaps treatment of fish poisoning cases. Copies of questionnaires prepared by marine biologists and physicians familiar with the fish poisoning problem accompany this publication. These questionnaires are designed to obtain specific information which we trust will lead to significant advances in our present knowledge of certain aspects of the problem.

The principal objectives of our epidemiological survey are as follows:

1. To determine areas affected by various types of fish poisoning and to correlate outbreaks with environmental conditions where possible;
2. To assemble information to aid in distinguishing the various types of fish poisoning, so that each might be studied separately;
3. To obtain information on fluctuations in toxicity of fishes in specific areas so that patterns might be correlated with changes in the local environment in order to supply clues as to the basic causes of fish poisoning;
4. To obtain a clearer picture of the syndromes found in various types of fish poisoning and thus to aid in establishing the nature of the toxin, the site of action, and the mode of action in humans;
5. To obtain information on native remedies whose efficacy in the treatment of fish poisoning might be investigated;
6. To disseminate all current information on fish poisoning to medical personnel throughout the affected areas of the Pacific in order to aid them in controlling the problem.

In surveys of the past, much valuable information has been lost because the native names of fishes involved in outbreaks of fish poisoning could not be equated to scientific or common names. As an integral part of this program, a compilation of native names of plants and animals of the Pacific islands, with their English common names and their scientific equivalents, is being made by the University of Hawaii. Such a compilation could prove itself to be a valuable asset not only to this program but to other workers in the Pacific area as well.

GENERAL INFORMATION ON FISH POISONING

This publication will deal only with those fishes that are poisonous to eat. It does not deal with fishes having venomous spines, or those dangerous because of their reputation for aggressive attacks on humans. Nor, among the fishes poisonous to eat, shall we consider those cases where the fish has become unfit to eat *after* being caught, due to bacterial contamination or putrefaction. (One type of poisoning, however, resulting from the rapid action of bacteria in scom-

broid fishes, will be included.) Fishes that are poisonous to eat may contain any of a number of toxins which may be found in the flesh, viscera, or other parts of the fish. In the types of fish poisoning to be considered, the toxin occurs naturally in the fish, either as part of its normal make-up or absorbed from its food or environment in a way that is not yet well understood.

Poisonous fishes are usually found in shallow-water areas around islands away from continental shores between latitudes within 30 degrees of the equator; they are most prevalent around certain islands of the Pacific Ocean and the Caribbean Sea. Fish poisoning is a particularly serious problem in some areas because it affects many of the fishes that are highly esteemed as food, and, since there are no distinguishing physical characteristics and since no simple test for the toxins exists, poisonous fishes cannot be readily identified. In addition, certain inconsistencies manifested by poisonous fishes make the problem particularly insidious in some areas. For example, both toxic and nontoxic fish of the same species may be found in the same area; or fish that are innocuous in one area may be highly toxic in another area a short distance away; occasionally, certain species of fish will suddenly become toxic in an area that has had no previous record of toxic fish; and of two closely related species found in the same area, one may be seasonally toxic and the other always toxic.

Fish poisoning of various types is an old problem in the Pacific, with records of outbreaks dating back to 1606 when the Spanish navigator Fernandez de Quiros and his crew were poisoned in the New Hebrides. Captain James Cook, the famed explorer, was likewise poisoned in the New Hebrides and in New Caledonia during his voyage to the Pacific in 1774. The journals of other early explorers, missionaries, and naturalists in the Pacific contain numerous accounts of episodes of fish poisoning which attest to a widespread existence of the problem prior to the present century. The occupation of many Pacific islands by military forces during World War II served to emphasize the gravity of the poison fish problem, for often these sojourners, lacking the native's knowledge of the potentially dangerous species of fish, were seriously afflicted.

Poisonous fishes have been classified into broad categories according to the species of fish implicated and the syndrome manifested in cases of human intoxications. Further research will be required to determine whether these categories validly reflect the action of a distinct toxin harbored by a clearly defined taxonomic group of fishes. The categories are listed below with current information on various aspects of fish poisoning obtained from the vast published literature on the subject, as well as from a large number of experienced correspondents in the Pacific area.

CIGUATERA POISONING
(Snappers, Groupers, Barracuda, Jacks, and Others)

Ciguatera is a term used to describe a disease that may result from the ingestion of any of a number of species of tropical marine reef fishes and is characterized by gastrointestinal and neurological symptoms. Ciguatera poisoning appears to present the most serious fish poisoning problem in the Pacific at the present time, primarily due to its widespread occurrence and the multiplicity of highly esteemed food fishes that may harbor the undetectable ciguatera toxin. The term is of Spanish origin and was first used in the Caribbean area to describe an illness caused by the ingestion of a marine snail, *Livona pica,* which the early Spanish settlers in Cuba called "cigua." The term gradually came into common usage as descriptive of a particular type of fish poisoning with a variable syndrome but with

certain characteristic symptoms which was encountered around islands in both the Caribbean and the Pacific.

Ciguatera is not a disease that is well understood, as only preliminary work has been done on the epidemiology, chemical isolation, identification, and biological origin of the toxin. However, based on this work, and upon observations of the symptoms displayed by persons afflicted by the disease, ciguatera appears to be distinct from the other types of fish poisoning to be described, with the possible exception of Gymnothorax poisoning.

A large number of species of fish have been implicated in outbreaks of ciguatera. These fishes are usually closely associated with reefs and shallow-water areas around islands; they include the snappers (Lutjanidae), groupers or sea basses (Serranidae), barracuda (Sphyraenidae), ulua, jacks, or pompano (Carangidae), surgeonfishes or tangs (Acanthuridae), and occasionally others. Some of the species involved in cases of ciguatera are illustrated in Figure 1.

It is generally agreed that fish causing ciguatera become toxic through factors existing in their environment; the most plausible theory in the light of existing evidence is that the toxin originates in a benthic organism (possibly a blue-green alga) and is transmitted to the various species of fish through the food chain in the process of normal feeding. It is possible, too, that a single fish might harbor more than a single type of toxin.

The results of a recent study show that no relationship exists between radioactivity and toxicity in snappers from the Marshall and Line islands, which is to be expected in the light of the past history of the poison fish problem in the Pacific.

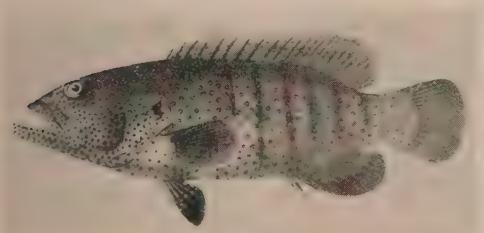
Fish causing ciguatera do not appear to be uniformly seasonally toxic, although some as yet uncorrelated fluctuations in the number of outbreaks of this disease in certain areas have occurred. It is not known whether these fluctuations are a reflection of the actual toxicity of the fishes in the area, or the effect of other factors such as fishing intensity. It has been observed that larger fish are generally more toxic than smaller fish of the same species in a given area.

In the Pacific, scattered reports over the past 350 years indicate that outbreaks of ciguatera have occurred in the following island groups: Ryukyu, Mariana, Caroline, Marshall, Gilbert, Ellice, Fiji, Samoan, Phoenix, Society, Tuamotu, and Marquesas, as well as in New Caledonia, the New Hebrides, and tropical Australia. In the past 20 years, fish caught in areas with no previous records of ciguatera have been reported as causing outbreaks of the disease. Such regions as the Line Islands, which had had no reports of ciguatera in over 100 years of recorded history, first reported toxic fish about 1940. These were followed by reports of ciguatera from Midway Island in 1944, Johnston Island about 1950, and the island of Oahu in the main Hawaiian Islands in 1957.

The clinical picture in ciguatera is often bizarre, and all patients do not display the same syndrome, even when all of the individuals have eaten portions of the same toxic fish. Ciguatera can usually be recognized on the basis of a few characteristic symptoms that may appear from a few minutes to 10 hours or more following the ingestion of a toxic fish. The first symptoms are usually experienced within about 3 hours after ingestion and consist of nausea and vomiting, followed by tingling and numbness about the lips, tongue, and throat. These may be followed by any of a multiplicity of other symptoms including abdominal pain and cramps, diarrhea, arthralgia, muscular weakness, incoordination, numbness and tingling of the extremities, malaise, chills, low-grade fever, and prostration. Hy-

a, Red snapper, *Lutjanus bohar* (Forskål)

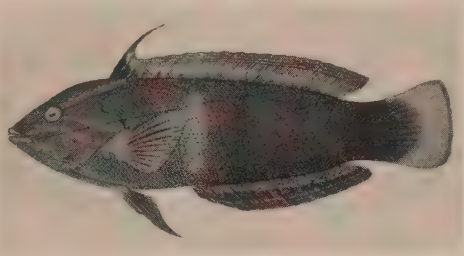
b, Gray snapper, *Lethrinus variegatus* Cuvier and Valenciennes



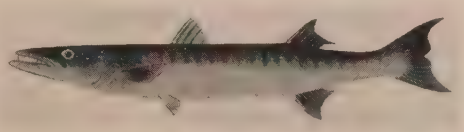
c, Grouper, *Cephalopholis argus* Bloch and Schneider

d, Jack, *Caranx lessonii* Cuvier and Valenciennes

e, Parrotfish, *Callyodon microrhinos* (Bleeker)



f, Wrasse, *Coris gaimardi* (Quoy and Gaimard)



g, Barracuda, *Sphyraena barracuda* (Walbaum)

h, Surgeon fish, *Acanthurus triostegus triostegus* (Linnaeus)

FIG. 1. Representative species from families of fishes which have been implicated in outbreaks of ciguatera. (All from Hiyama.)

potension, profuse sweating, dyspnea, restlessness, insomnia, headache, intermittent dizziness, dilatation of the pupils, ptosis, divergent strabismus with diplopia, reduced vision, dryness of the mouth, a metallic taste, and myalgia (particularly severe in the back and thighs) also occur. Hyperesthesia, urinary retention and diminished to absent knee and ankle reflexes have been reported. Patients often experience dysesthesia that consists of a confusion of temperature sensation; when touching a cold object, the patient reports that it gives the sensation of burning, tingling, or "dry ice," and hot objects feel cold. When tap water is swallowed, it often gives the sensation of being carbonated. In severe cases of ciguatera, shock, convulsions, muscular paralysis, and death may occur.

It appears as though the body does not rapidly neutralize or eliminate ciguatera toxin, for characteristically, the recovery period is quite prolonged. In moderate cases of poisoning, most of the symptoms subside in 24 hours, with the exception of the muscular weakness, tingling, and numbness which may last from 4 to 7 days. During the recovery phase some patients report a vague neuralgic pain about the teeth, and intense itching, especially on the palms of the hands and soles of the feet. In severe cases, symptoms may persist for several weeks or months, and complete recovery from sensory disturbances, weakness, and weight loss may require much longer.

An attack of ciguatera does not impart immunity; on the contrary, patients who have been poisoned previously report a mild recurrence of symptoms after eating a potentially toxic fish, while others who have never been poisoned experience no symptoms when eating the same fish.

Usually treatment recommended for ciguatera is symptomatic, consisting initially of emptying the digestive tract by means of gastric lavage, emetics, and saline purges. A variety of other treatments have been suggested and tried with varying palative effect, but none have been consistently successful; these include injections of calcium gluconate, injections of vitamin B complex, infusions of glucose in normal saline, phenobarbital, belladonna, paregoric, codeine, and aspirin. In one outbreak of ciguatera involving five persons, procain hydrochloride infusions were inadvertently substituted for the calcium gluconate recommended and the patients displayed a regression of symptoms and general improvement. Subsequent administration of calcium gluconate in place of the procain hydrochloride resulted in a recurrence of symptoms; reinstitution of the procaine treatment again resulted in abatement of the symptoms followed by complete recovery of the patients within a week.

A recent outbreak of ciguatera on Guam was caused by the ingestion of a portion of a 53-pound barracuda. In the case of one of the victims, a 19-year-old female, severe depression and paralysis were experienced, and treatment with hydrocortisone, tensilon, and neostigmine seemed to have a beneficial effect.

Recent research in Japan and at the University of Hawaii indicates that the toxins extracted from barracuda, snappers, and pompano are similar and probably identical. These toxins produced ciguatera symptoms in man and laboratory animals. The toxin from the red snapper, *Lutjanus bohar*, from the Line Islands has been most thoroughly studied; this thermostable toxin is initially soluble in 90 and 100 per cent ethanol, acetone, chloroform, and diethyl ether, and insoluble or only slightly soluble in water, butanol, benzene, and petroleum ether.

No simple test for ciguatera toxin exists; a quantitative bioassay has been developed which entails the intraperitoneal injection of an alcohol-ether extract of fish flesh into

laboratory mice. The method of bioassay for ciguatera utilizing an aqueous extract of fish flesh or viscera is of doubtful validity; therefore, prior surveys on the geographical distribution and relative toxicity of fishes causing ciguatera employing this bioassay are of questionable value.

TETRAODON POISONING
(Puffer Fishes)

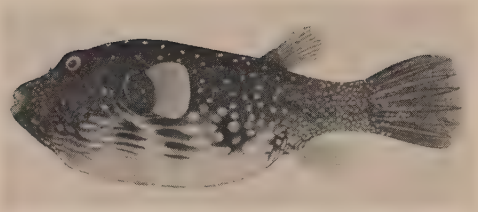
Tetraodon poisoning is caused by fishes of the family Tetraodontidae, commonly known as pufferfish, balloonfish, blowfish, or globefish; these names are descriptive of a peculiar habit these fish have of inflating themselves with air or water to assume a spheroid shape. Pufferfish are peculiar looking fish which lack pelvic fins and are generally feeble swimmers. A large number of species occur in the tropical Indo-Pacific area, and cases of poisoning from their ingestion have been reported from scattered points throughout their range. Most of the outbreaks have occurred in Japan where the pufferfish (called *fugu* by the Japanese) is considered a delicacy. Normally, special care is taken in its preparation to render it innocuous; however, carelessness in cleaning and preparation results in many deaths each year in Japan. The toxin appears to be concentrated in certain organs and tissues; the ovaries are said to be always poisonous, while the liver, bile, skin, and flesh may occasionally be toxic. It is not known if all species of puffer fishes are toxic, or if all those that are toxic contain the same toxin, as only a few species, particularly those esteemed as food, have been studied.

In addition to the Tetraodontidae, there are a number of other closely related families of the order Tetraodontiformes (Plectognathi) that have been reported toxic when eaten; however, the reports are neither numerous enough nor sufficiently detailed to determine whether they contain a toxin identical or similar to that found in the puffers. These other closely related families include the filefishes (Monocanthidae), the trunkfishes or boxfishes (Ostraciidae), the triggerfishes (Balistidae), the sharp-backed puffers (Canthigasteridae), and the spiney puffers (Diodontidae).

Pufferfish (Tetraodontidae) may be highly toxic and the onset of symptoms may develop within 10 minutes after ingestion, or more than 3 hours may pass before the first symptoms are experienced. Initial symptoms include tingling of the lips, tongue and fingertips, followed by progression of the numbness which may involve the entire body, and extreme weakness associated with nausea, vomiting, headache, profuse sweating, subnormal temperature, hypersalivation, dysphagia, dysphonia, dyspnea and a constricting sensation in the chest. In severe, acute cases, the patient rapidly develops a weak, rapid pulse, hypotension, aphonia, marked dyspnea, cyanosis, and an ascending paralysis with death resulting from respiratory failure. Death usually occurs within 24 hours after ingestion; if the patient survives 24 hours, the prognosis is good.

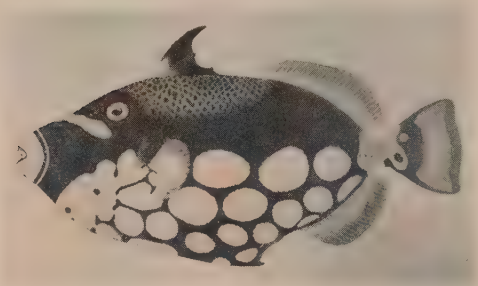
The toxin from pufferfish has been isolated by Japanese workers who have called it "Tetrodotoxin." A number of investigators have studied its chemistry and toxicology. Tetrodotoxin has a curare-like action on the neuromuscular junction; it depresses conductivity in somatic motor and sensory nerves and in sympathetic fibers, and depresses the medullary centers.

No specific antidote has been developed for tetraodon poisoning; the treatment recommended is symptomatic. Japanese workers who have had extensive experience in the treatment of tetraodon poisoning recommend the following: nikethamide, metrazol, or other suitable respiratory stimulants to counteract respiratory depression together with artificial respiration in the event of respiratory failure; administration of vasopressin, and rapid digitalization to



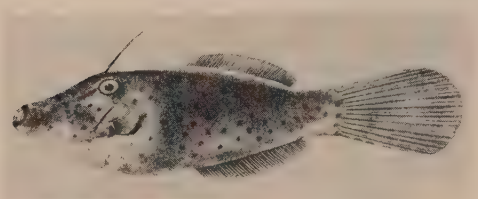
a, Smooth puffer, *Arothron hispidus* (Linnaeus) (From Hiyama)

d, Spiny puffer, *Diodon histrix* Linnaeus (From Jordan and Evermann)



b, Triggerfish, *Balistoides conspicillum* Bloch and Schneider (From Hiyama)

e, Sharp-backed puffer, *Canthigaster cinctus* (Richardson) (From Jordan and Evermann)



c, Filefish, *Alutera scripta* (Osbeck) (From Day)

f, Boxfish, *Ostracion lentiginosus* (Bloch & Schneider) (From Jordan and Evermann)

FIG. 2. The pufferfish implicated in cases of Tetraodon poisoning and representatives of some closely related families of plectognath fishes that have been reported to contain toxic species.

correct hypotension and circulatory collapse; and physostigmin in an attempt to reverse the neuromuscular block.

Tetrodotoxin appears to be endogenous, being stored primarily in the gonads. In at least one species of pufferfish, *Fugu niphobles,* the fish may eat some of its own recently spawned toxic eggs, which suggests a mechanism by which the toxin might disburse to the other organs and tissues of the fish. There is no simple chemical test that can be used to assess the toxicity of a pufferfish. The standard bioassay requires extraction of the water soluble toxin and intraperitoneal injection of this aqueous extract into laboratory mice.

ELASMOBRANCH POISONING
(Sharks)

Poisoning from the ingestion of the flesh and liver of tropical sharks has occurred in scattered localities in the Pacific. The three species of tropical sharks implicated in outbreaks of elasmobranch poisoning are the black-tipped sand shark, *Carcharhinus melanopterus* (Quoy and Gaimard), the great white shark, *Carcharodon carcharias* (Linnaeus), and the hammerhead shark, *Sphyrna zygaena* (Linnaeus). Other species of sharks from temperate and arctic waters have also been reported to cause poisoning in humans and domestic animals.

FIG. 3. *Carcharhinus melanopterus* (Quoy and Gaimard), the black-tipped sand shark that has been implicated in cases of Elasmobranch poisoning. (From Jordan and Evermann.)

Ingestion of the flesh of tropical sharks has caused only mild gastrointestinal upset; however, severe poisonings have been recorded from the ingestion of the liver. The ingested liver of a toxic shark can produce symptoms within 30 minutes. These include headache, nausea, vomiting, diarrhea, aching joints, heaviness of the limbs, prostration, delirium, feeble pulse, diaphoresis, respiratory distress, thoracic pain, burning sensations of the tongue, throat, and esophagus, and tingling of the lips and extremities. Twitching of the eyelids, pruritus, and desquamation have also been reported. In severe cases coma and death may result.

Very little is known of the chemical nature or physiological action of the elasmobranch toxin and, therefore, the recommended treatment is symptomatic. Evacuants may be helpful, if administered promptly.

Regarding the origin of the toxin, one investigator claims that sickness is due to an overdose of vitamin A, while others believe that it is related to ciguatera, perhaps the result of sharks ingesting fish containing ciguatera toxin.

Elasmobranch poisoning appears to be rather uncommon in the Pacific. Reports have been received of outbreaks of elasmobranch poisoning in Samoa, the Line Islands, the Society Islands, New Caledonia, and the southern Marshall Islands. Outbreaks have occurred elsewhere in the tropics, in Mauritius in the Indian Ocean, and in Bermuda in the Atlantic.

GYMNOTHORAX POISONING
(Moray Eels)

Moray eels are abundant throughout Oceania and some species are considered excellent food fishes.

At least seven species of moray eels from the Marshall, Mariana, and Samoan islands have been reported as causing an intoxication in humans called gymnothorax poisoning. These species, all of the genus *Gymnothorax,* cause a poisoning that resembles ciguatera in many respects. As in ciguatera, a species of moray eel that is toxic in one locality may be edible in another, and the larger specimens appear to contain more toxin than the smaller ones.

The syndrome of gymnothorax poisoning resembles that of ciguatera but with the following additional symptoms: excessive mucous production, trismus, laryngeal spasm, the absence of thoracic respiration with pronounced abdominal breathing, purposeless movements, and an extended period of absent reflexes. Rapid respiration, rapid pulse, and a high fever may develop in gymnothorax poisoning cases. In gymnothorax poisoning the onset is generally more rapid and more violent than in cases of ciguatera, and the paralysis, convulsions, and coma are

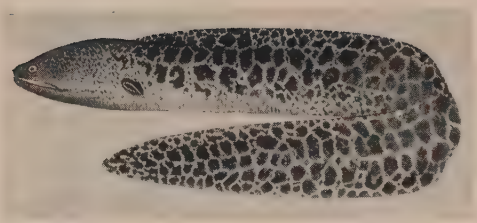


FIG. 4. *Gymnothorax flavimarginatus* (Rüppell), a moray eel which has been implicated in cases of Gymnothorax poisoning. This species caused illness in 57 men on Saipan after they had eaten of a 6-foot specimen. (From Hiyama.)

more striking. The clinical information on gymnothorax poisoning is based on but two outbreaks in the Marshall and Mariana islands.

No specific antidote has been developed for gymnothorax poisoning; the treatment recommended is symptomatic. Intravenous injections of 1.5 to 2.5 gm. of 10 per cent calcium gluconate and 10 per cent calcium chloride have relieved symptoms in some patients, but failed to do so in others.

Gymnothorax toxin is reported to be soluble in water and acid methanol, resistant to destruction by pH and temperature changes, and is dialysable. A bioassay of questionable validity for the toxin consists of intraperitoneal injection of a crude extract into laboratory mice.

SCOMBROID POISONING
(Tuna and Mackerel-like Fishes)

Scombroid poisoning is an allergy-like intoxication caused by the bacterial action of improperly stored tuna, skipjack, bonito, and other mackerel-like fishes which are highly esteemed as food fishes throughout the Pacific as well as in other areas. These fishes become dangerous to eat when certain strains of the bacterium, *Proteus morganii*, act on a naturally occurring substance in scombroid fish flesh called histidine. This action causes the production of histamine and a histamine-like substance called saurine without producing the usual signs of putrefaction. This bacterial action may be extremely rapid in warm climates if the fish is not properly refrigerated.

The histamine and saurine produced may cause a severe allergy-like reaction in man upon the ingestion of scombroid fish flesh containing these products. The presence of these toxic substances is sometimes detected upon initial ingestion by a "sharp" or "peppery" taste. Symptoms develop within a few minutes to 3 hours and are often sudden in onset. These include erythema of the face and upper part of the body, severe occipital headache, giant urticaria, conjunctivitis, and periorbital edema, edema of the lips, tongue, and throat, respiratory distress, tachycardia, abdominal pain, malaise, generalized weakness and giddiness. Fever and mild diarrhea occur in a few cases as does nausea, but victims rarely vomit. A few cases have been reported in which the patient has gone into shock followed by death; however, the acute symptoms usually persist for from 8 to 12 hours after which the patient experiences a rapid recovery.

The treatment recommended is immediate evacuation of the stomach contents followed by the administration of antihistaminic drugs.

Tuna and mackerel-like fishes are primarily pelagic fishes and are widely exploited by the commercial fishermen in the Pacific.

FIG. 5. *Katsuwonus pelamis* (Linnaeus), a species of tuna implicated in cases of scombroid poisoning.

It should be emphasized that this group of fishes contains no known endogenous or acquired toxin while in the sea; they become toxic only through improper handling after capture. Some evidence exists that a similar condition may develop in other groups of fishes, including the dolphins or mahimahi (Coryphaenidae).

HALLUCINATORY MULLET POISONING

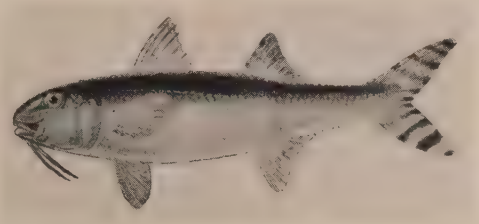
(Mullet, Surmullet, Rudderfish, and Possibly Others)

Hallucinatory mullet poisoning is a seasonal condition reportedly caused by the ingestion of certain fishes on at least three Pacific islands. Outbreaks of this malady have occurred intermittently for over 60 years during the months of June, July, and August in restricted localities on the northeastern coast of the island of Kauai, and on the southern coast of the island of Molokai in the Hawaiian archipelago. A similar condition has been reported from Norfolk Island in the southwest Pacific; however, insufficient information exists to determine if the diseases produced in these two widely separated areas are identical.

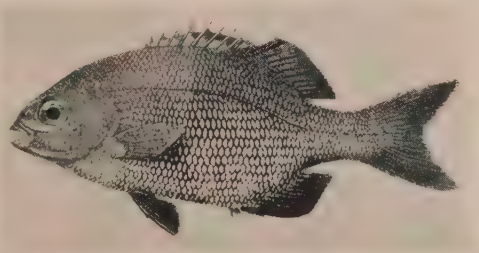
Four species of fish, representing two families, are commonly implicated in intoxications in the Hawaiian Islands. These are the mullet, *Mugil cephalus* Linnaeus, and *Neomyxus chaptalli* (Eydoux and Souleyet), and the surmullet or goatfish, *Mulloidichthys samoensis* (Günther), and *Upeneus arge* (Jordan and Evermann). In addition, the surgeonfish or tang, *Acanthurus sandvicensis* (Streets), and the rudderfish, *Kyphosus cinerascens* Forskål, have occasionally been implicated in a similar type of intoxication in these same localities in the Hawaiian Islands. The fish from Norfolk Island reported as causing intoxications is the rudderfish, *Kyphosus fuscus* (Lacépède).

The symptomatology is only known for the Hawaiian intoxications. The onset of symptoms has been reported to be from 10 minutes to 2 hours after ingestion; symptoms usually persist for a few hours, but occasionally have been reported as lasting up to 24 hours. The toxin appears to involve primarily the higher nervous system and produces dizziness, loss of equilibrium, lack of coordination, hallucinations, and mental depression. If the symptoms begin while the patient sleeps, he develops nightmares. Other symptoms vary, with some victims re-

a, Mullet, *Mugil cephalus* Linnaeus (From Jordan and Evermann)



b, Surmullet, *Upeneus arge* (Jordan and Evermann) (After Jordan and Evermann)



c, Rudderfish, *Kyphosus cinerascens* Forskål (From Day)

FIG. 6. The species most commonly implicated in outbreaks of hallucinatory mullet poisoning from Hawaii.

porting an itching or burning of the throat immediately after eating, others a muscular weakness and even a partial paralysis of the legs. Nausea, abdominal discomfort, or diarrhea are rarely reported. Reports on Norfolk Island intoxications merely state that ingestion of the implicated rudderfish produces terrible nightmares.

All reports of this poisoning indicate that it is mild and nonfatal. Since no specific antidote has been developed, treatment is symptomatic.

The toxin causing this poisoning appears to be distributed throughout the body of the fish; some reports claim that it is concentrated in the head. Nothing is known of the chemistry or toxicology of the poison. There are indications that it is not of bacterial origin.

CLUPEOID POISONING
(Herring-like Fishes)

Although clupeoid poisoning does not appear to be widespread in the Pacific, it is a category of fish poisoning that is distinct from the others described and involves a very potent toxin. This type of poisoning is caused by the ingestion of a variety of clupeoid fishes including *Clupea fimbriata, Clupea thrissa,* and *Melleta venenosa*. Outbreaks of clupeoid poisoning have been reported from Tahiti, New Caledonia, Fiji, and Indonesia. In Fiji, five deaths in 1955 resulted from the ingestion of a local herring. Apparently two closely related species of herring were involved in outbreaks of clupeoid poisoning in Fiji; both are known locally as "Daniva" (=*Sardinella* spp.?). One species is reported to be toxic throughout the year, and the other only during and after the rising of the "palolo" worm, a marine worm that swarms at a certain phase of the moon in October and November and is esteemed as food by the local inhabitants. Although the palolo worms are nontoxic, one species of herring, as well as other fishes, appear to become toxic for a few weeks during and after the swarming period of the worms.

FIG. 7. *Clupea fimbriata* (Cuvier and Valenciennes), a species of herring implicated in outbreaks of clupeoid poisoning. (From Weber and de Beaufort.)

There is little published clinical information on clupeoid poisoning from the tropical Pacific. Symptoms reported include nausea, vertigo, itching, abdominal pain, tenesmus, tachycardia pulse, cold sweats, dyspnea, cyanosis, dilated pupils, coma, convulsions, and at times death within a half-hour of ingestion. Survivors may be jaundiced.

Nothing is known of the nature of the toxin in clupeoid poisoning. No specific antidote has been developed and the treatment is symptomatic.

OTHER TOXIC MARINE ORGANISMS

Numerous other marine organisms other than fin fishes from the tropical Pacific have been reported poisonous to eat and they are of particular interest because of their possible relationship to the categories of fish poisoning presently being investigated. There have been no reports of extensive poisoning in marine organisms other than fish, but scattered reports from various locations throughout the Pacific indicate that intoxications have occurred from the ingestion of sipunculid worms, octopuses, sea urchins (*Tripneustes*), gastropods (*Trochus*), coral-boring pelecypods, crabs, and lobsters. Seasonal paralytic shellfish poisoning of the

type that is found on the shores of North America and Europe has not been reported from the tropical Pacific. In addition to invertebrates, reports have been received of serious intoxications from the ingestion of the flesh of marine turtles including the hawksbill turtle, *Eretmochelys imbricata* (Linnaeus), the green sea turtle, *Chelonia mydas* (Linnaeus), and the leatherback turtle, *Dermochelys coriacea* (Linnaeus). It is expected that during the course of investigations on poisonous fishes further information will be obtained on these and other marine organisms that are occasionally toxic.

It is hopefully assumed that those closest to the problem already share our interest and desire to obtain more exact information on fish poisoning so that progress can be made toward better recognition and control of human intoxications. It is the hope of the scientists in this investigation that you will share in this unique opportunity to cooperate in our research by contributing your first-hand information by means of our questionnaires.

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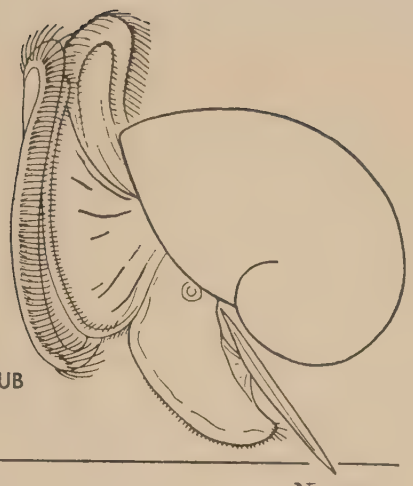

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On *Cypraea tigris schilderiana* Cate

by

Alison Kay

Department of General Science, University of Hawaii, Honolulu, Hawaii

(This paper is Contribution No. 156 from the Hawaii Marine Laboratory)

(Plate 8 and 2 Textfigures)

Cypraea tigris schilderiana from the Hawaiian Islands has recently been described by Cate (1961b). The purpose of this paper is to contribute further measurements and records of C. tigris both in the Hawaiian Islands and elsewhere in the Pacific, and to suggest three matters which merit further consideration: 1) the possible existence of C. tigris schilderiana outside the Hawaiian Islands; 2) the difference in habitat exhibited by C. tigris in the Hawaiian Islands and elsewhere in the Pacific; and 3) the variability in size of the subspecies within the Hawaiian chain of islands.

Shell measurements of Cypraea tigris were obtained from material in the Bernice P. Bishop Museum in Honolulu, and from numerous private collections in the State of Hawaii. A detailed geographical analysis of the specimens examined is summarized in Table 1. Measurements of length, breadth, and height were made to the nearest millimeter with vernier calipers, and the means and standard deviations for these measurements calculated. The shells were separated into three groups: 1) those from the Hawaiian Islands; 2) those from Johnston Island; and 3) those from other Pacific areas. The 75 percent rule for subspecies as set forth by Mayr, Linsley, and Usinger (1953) was applied by use of calculations of the coefficient of difference.

Figure 1 (see Plate 8), a length-frequency histogram, shows that whereas specimens of Cypraea tigris throughout the Pacific range in length between 51 and 147 mm., the majority of Hawaiian examples is longer than 100 mm., and the majority of those from other areas of the Pacific, with the exception of Johnston Island, is less than 100 mm. in length. Of the 403 individuals measured, only two specimens from the Hawaiian Islands were less than 95 mm. in length, and only nine from other Pacific areas excluding Johnston Island were greater than 95 mm. in length. The mean length of the Hawaiian specimens measured was 117 mm.; the mean length of those from other Pacific areas excluding Johnston Island was 77 mm.

The difference in size between specimens of Cypraea tigris from the Hawaiian Islands and Johnston Island, and those from elsewhere in the Pacific is further emphasized by comparisons of breadth and height (Table 1).

The figures for the coefficient of difference for the three linear dimensions of 1.9 for length, 1.9 for breadth, and 2.0 for height indicate that 95 percent of the Hawaiian population is different from the Pacific population (excluding Johnston Island) for these dimensions. Following Mayr, Linsley, and Usinger (1935), the Hawaiian population, considered only from the

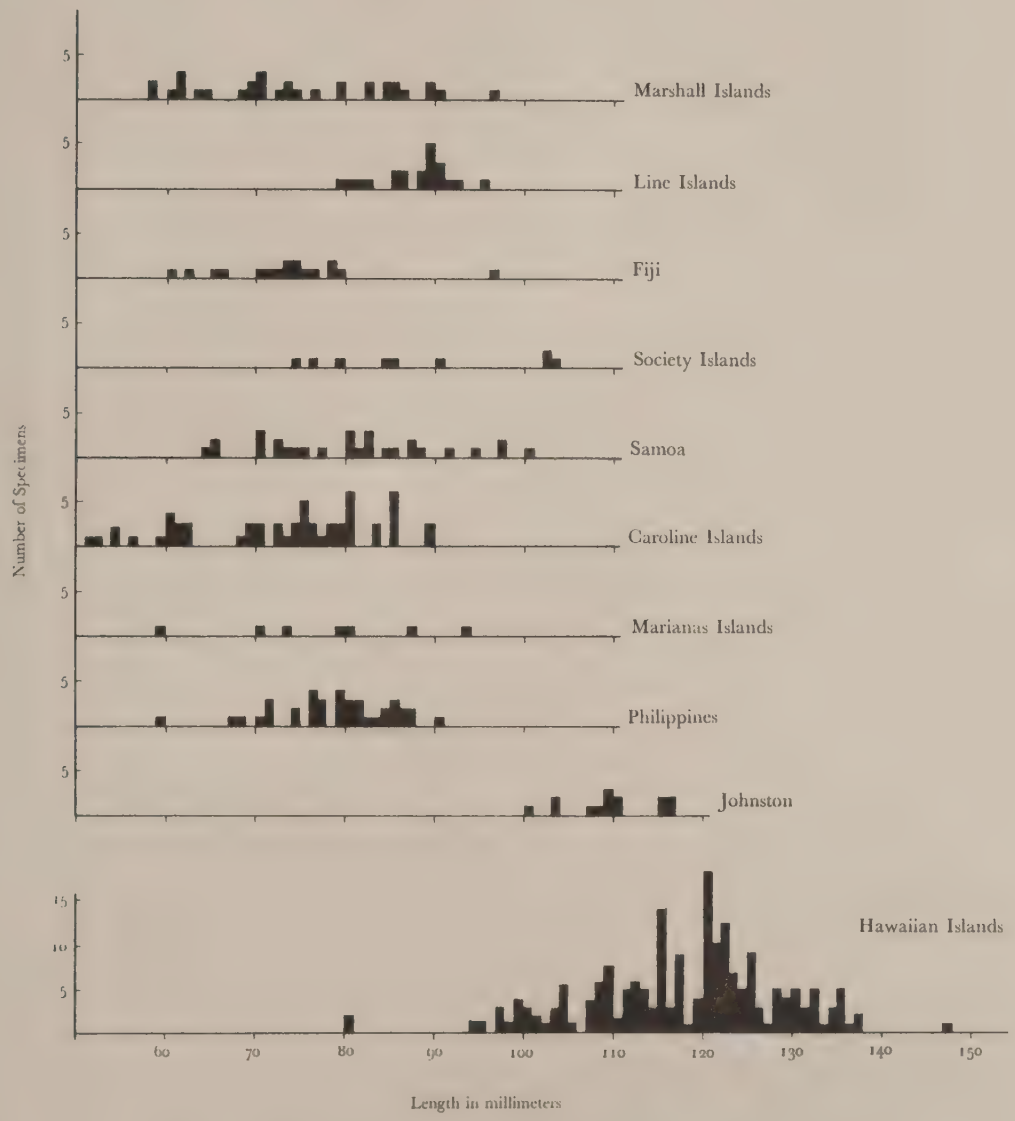

Figure 1: Length-frequency histogram showing the variations in size of *Cypraea tigris* in the Hawaiian Islands and other Pacific areas

Table 1: Geographical and Statistical Summary

Locality	No. of Specimens	Length			Breadth			Height		
		Range[1]	Mean[1]	S. D.[2]	Range[1]	Mean[1]	S. D.[2]	Range[1]	Mean[1]	S. D.[2]
Hawaiian Is.	198	80-147	117	10.1	56- 96	80	7.14	39- 81	64	6.3
Johnston Is.	14	100-116	100	—	72- 80	77	—	55- 65	59	—
Pacific	205	51-103	77	10.2	37- 77	54	6.1	28- 57	43	5.7
Marshall Is.	32	58- 96	76	—	42- 67	53	—	33- 57	43	—
Line Is.	21	79- 95	91	—	53- 65	54	—	41- 52	41	—
Samoa	29	64-100	77	—	43- 66	54	—	35- 54	44	—
Fiji	17	60- 96	73	—	42- 64	51	—	36- 47	41	—
Marianas Is.	7	59- 93	77	—	43- 63	54	—	35- 49	43	—
Caroline Is	52	51- 90	74	—	37- 65	49	—	28- 50	39	—
Society Is.	9	74-103	88	—	50- 77	60	—	40- 56	49	—
Philippines	38	59- 90	78	—	43- 59	54	—	35- 49	44	—

[1] Measurements in millimeters [2] Standard deviation

standpoint of linear dimensions, may be separated as a subspecies. No statistically significant differences were found for calculations of length/breadth and length/height ratios.

It is apparent from the data (Table 1 and fig. 1, Plate 8) that samples of Cypraea tigris from Johnston Island, which is 450 miles south of the Hawaiian Islands, fall within the size range of the Hawaiian specimens. The mean dimensions of the Johnston Island specimens are, however, slightly smaller than those of the Hawaiian sample. Because of the small sample of Johnston Island material, statistical tests for this group have not been included. However, it should be pointed out that the shells from Johnston Island resemble those from the Hawaiian Islands in color and lack a marginal callus, characters which Cate (1961b) considers distinctive of the Hawaiian subspecies. An hypothesis accounting for the occurrence of a population of C. tigris at Johnston Island similar to the Hawaiian population has been put forward elsewhere (Kay, manuscript in press).

A survey of the literature and discussions with shell collectors in Hawaii disclose that the habitat of Cypraea tigris in the Hawaiian Islands is in contrast to its habitat elsewhere in the Pacific. In the Hawaiian Islands C. tigris is collected from depths of eight to ten feet and deeper (with one or two records of collection at depths of four to eight feet) where it is found beneath dead coral and on basalt boulders and spits. In other areas of the Pacific the species has been described as occurring exposed on the surface of large coral heads at Biak in the Marshall Islands (Bayr and Neurohr, 1946), in association with living coral heads on reefs between the tide marks in American Samoa (Ingram, 1939), and on various lagoon and seaward reefs in the Pacific (Demond, 1957).

Specimens of Cypraea tigris in the Hawaiian Islands vary in size with both depth and locale. Cate (1961a) has noted that "the smaller shells seem to come from the shallower localities in the southern end of the range, medium-sized ones from the intermediate depths of mid-range, and the largest known in the world from deep-water Oahu stations." Data confirming Cate's observations are presented in figs. 2 and 3. Although measurements for specimens from the Island of Hawaii are few and inconclusive due to the unavailability of records at the present time, the data from the islands of Kauai, Oahu, and Maui indicate a tendency for the larger specimens to be more abundant around the northern islands, with the exception of the extremely large specimens which were collected from deep waters off Oahu. It is also apparent that depth of collection varies with the islands; individuals from Kauai, the northernmost of the main islands in the Hawaiian chain, having been found at depths of 25 to 40 feet, while the islands to the south yielded collections from progressively shallower water.

Two specimens of Cypraea tigris have been examined from islands to the north of Kauai. It is noteworthy that both, one from Midway and the other from Kure, fall into the size range of material from the Pacific, being 63 mm. and 75 mm. in length, respectively. They also exhibit the callus and color pattern characteristic of the Pacific type. The dimensions of these specimens have not been included in the analysis of data, because of their apparent similarity to individuals from the other Pacific areas.

Another interesting feature concerning Cypraea tigris in the Hawaiian Islands is the shortage of collections of juveniles. There are only three known juveniles from Hawaii, all three collected by Dr. C. M. Burgess. Other

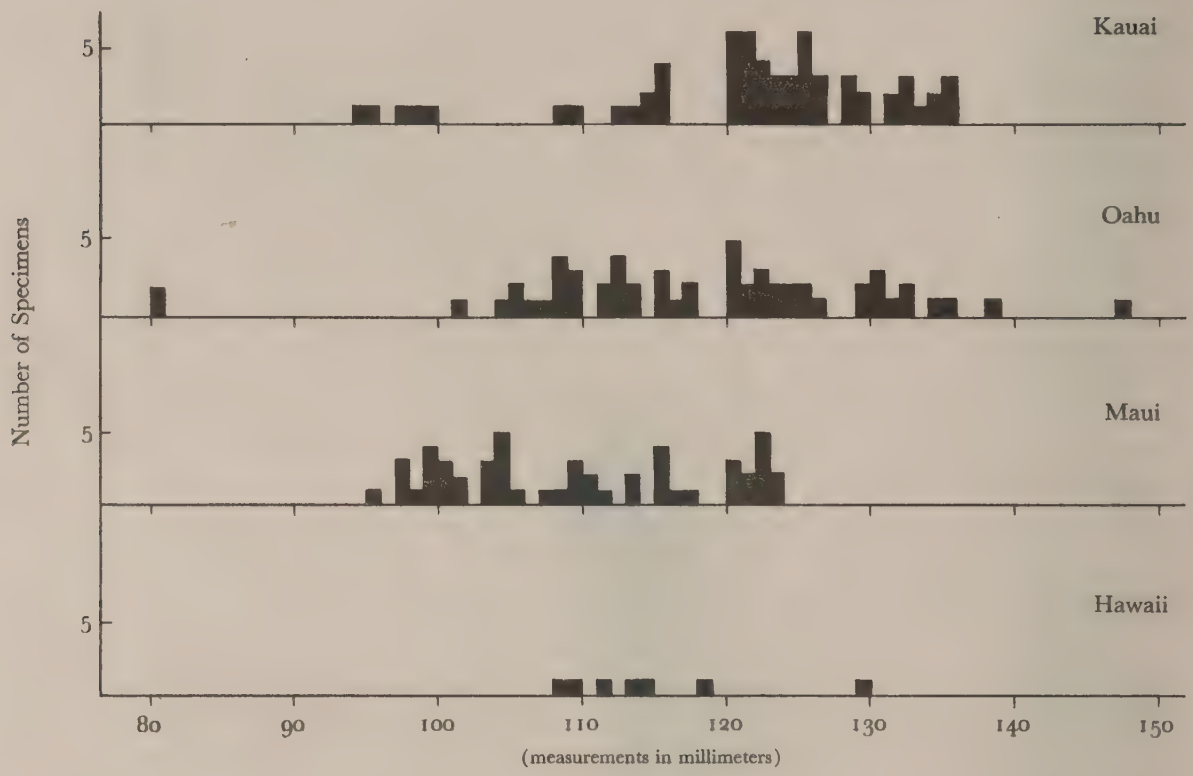

Figure 2: Length-frequency histogram showing the relationship between length and island for *Cypraea tigris* in the Hawaiian Islands

shell collectors with wide collecting experience have remarked that they have been unable to find juveniles of this species, although they are well known for other species of Cypraea.

The history of Cypraea tigris in the Hawaiian Islands is sparse. Cate (1961a) has noted that Schilder (1933) considers early records of Garrett (1879), Baldwin (1898), and Hidalgo (1906) doubtful, and Cate regards the first authentic record to be that of a specimen collected in 1929 and recorded by Harris (1935). Cate's (1961a) reference to a record cited by Martens and Langkavel (1871) is apparently an error; the reference quoted refers to Terebra tigrina from the Kingsmill Islands. Martens and Langkavel (1871) do not refer to C. tigris from the Hawaiian Islands.

Two early reports of Cypraea tigris in the Hawaiian Islands have recently come to the author's attention. The naturalist on the Portland and Dixon voyage to Hawaii mentions "...beautiful shells such as Cypraea tigrina..." from the Hawaiian Islands (Dixon, 1789). Another record is that of W. H. Pease, who described a specimen of "...Cypraea tigris from your island (Hawaii) five inches in length and over nine inches around the thickest part..." in a letter to Andrew Garrett dated March 5, 1857 (Manuscript Collection, B. P. Bishop Museum, Honolulu). As the dimensions mentioned by Pease fall well within the size range of the Hawaiian specimens, it would appear that C. tigris had been collected in the Hawaiian Islands prior to the 20th century, although Pease notes in the same letter, "...I never obtained but few."

While there are no records of Cypraea tigris in the Hawaiian archaeological collections of the B. P. Bishop Museum or among fossils from the late Pleistocene, there is one fossil example of C. tigris which has been tentatively dated as Recent. The shell was dredged from not more than four feet below the surface of a reef near Kaaawa, Oahu, in 1948 (R. Gage, personal communication). Ostergaard (personal communication) observes that the specimen cannot be assigned an age comparable to that of the fossiliferous limestone of Oahu (usually considered Late Pleistocene) because of its position below the surface of the reef.

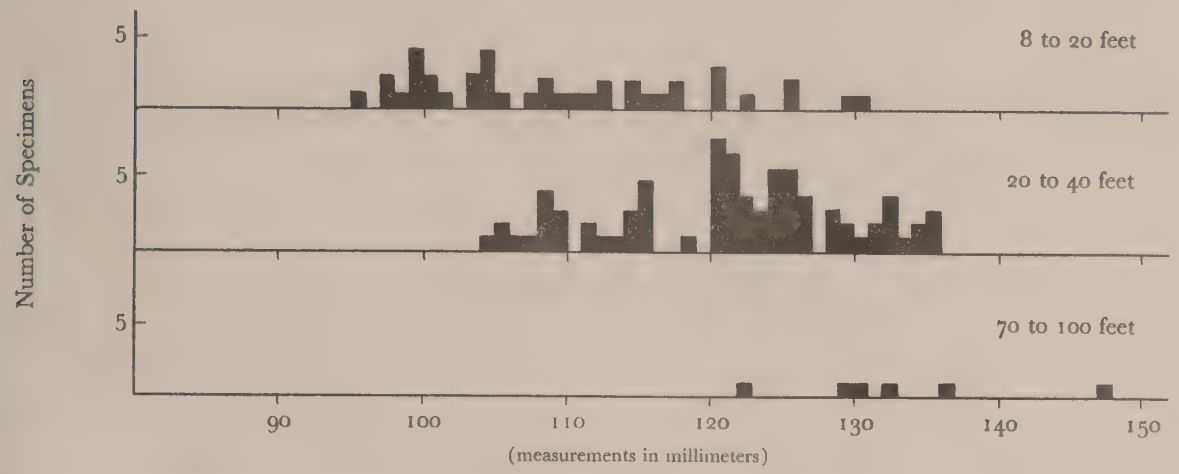

Figure 3: Length-frequency histogram showing the relationship between length and depth of occurrence of *Cypraea tigris* in the Hawaiian Islands

Conclusions

The data presented here, although somewhat scanty, are of interest not only with respect to studies of Cypraea, but as materials for speciation studies of marine invertebrates. It is well known that isolated areas and those near the periphery of a range are frequently inhabited by populations with distinctive biological characteristics. The existence of a distinctive population of C. tigris in the Hawaiian Islands, an area isolated from other Pacific islands by expanses of deep water and forming the eastern periphery of the range of C. tigris in the Indo-West Pacific is, therefore, not surprising. That the distinctive features of peripheral populations are correlated with ecological factors is also a generalization, but there is little information on the effects of ecological factors. We are particularly handicapped by the dearth of information and lack of experimental studies concerning early stages in life history. These data accumulated for adult C. tigris are, nevertheless, useful in that they focus on two major questions: 1) how are we to account for the large size of C. tigris in the Hawaiian Islands? and 2) is the size difference exhibited by the Hawaiian population genotypic or phenotypic?

Increased size in various animals has long been associated with low temperatures (Bergmann's Rule). Since surface temperatures of Hawaiian waters are at least 1.5°C. cooler than the lowest surface water temperature of other areas from which we have records of C. tigris (Sverdrup, et al., 1946), temperature must be considered as a possible factor affecting the size of individuals in the Hawaiian population of C. tigris. But the picture is complicated by the apparent presence of the Pacific type of C. tigris at Midway and Kure which have the lowest surface water temperatures in the Hawaiian chain, and of the Hawaiian type at Johnston Island which has a higher mean annual surface water temperature than that of the Hawaiian Islands (Sverdrup, et al., 1946). The occurrence of specimens in relatively deep water in the Hawaiian Islands compared with their existence in shallow water elsewhere in the Pacific, and variation in depth at which the species is found within the Hawaiian Islands is another complicating factor. Is there a relationship between water temperature and depth which would account for the observations? Or is there a factor associated with a different habitat? The Hawaiian Islands are high islands with reef formation and constitution considerably different from the atolls elsewhere in the Pacific. Are there differences in food and feeding habits? Is larval population density a factor affecting adult size?

The question as to whether the differences in size of Cypraea tigris are phenotypic or genotypic is equally provocative. It has been pointed out that slight geographical variation has been demonstrated in many marine invertebrates and the suggestion has been made that this sort of variation is a response to local environmental conditions (Mayr, 1954). The variation in size and depth of occurrence exhibited

by C. tigris within the Hawaiian Islands is probably best considered an example of this type of variation. Is the presence of the Hawaiian type of C. tigris at Johnston Island, where there is a higher mean annual surface water temperature and an atoll-type environment, indicative of a genotypic difference when the pronounced difference in size of the individuals in the Hawaiian population is considered?

It is evident that there are no simple answers to the two questions posed above. That we are able to enumerate some of the factors and speculate on their possible effects is encouraging. With the further accumulation of ecological data and emphasis on experimental work we may in time contribute further information toward an elucidation of the factors affecting speciation in marine invertebrates.

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I would like to acknowledge the patience and help of the many collectors in Hawaii who have contributed their collections of Cypraea tigris for measurement and their observations for analysis. I am particularly grateful to Dr. Yoshio Kondo of the B. P. Bishop Museum in Honolulu, to Dr. C. M. Burgess, Mr. and Mrs. Arch Harrison, Mr. Harold Jewell, and Mr. and Mrs. James Dennis of Honolulu, and to Mr. John Duarte of Kauai.

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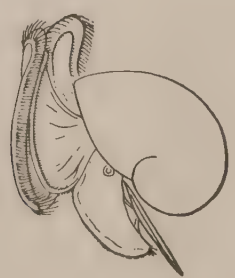

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New Arrangement of Interrenal and Chromaffin Tissues of Teleost Fishes

Abstract. An unusual morphologic arrangement of interrenal and chromaffin tissues has been observed in two families of teleost fishes. In most teleosts in which these cell types occur together in association with the postcardinal veins and their branches, the chromaffin cells are located between the lumen of the vein and the interrenal cells. This typical relationship is reversed in some of the Labridae and Scaridae.

The interrenal gland of teleost fishes, located within the anterior or head kidney, appears to be homologous to the adrenal cortex of mammals (*1*, *2*). Chromaffin cells like those of the adrenal medulla, which react with chromic acid, are also found in the anterior kidney. These cell types were delineated first by Giacomini in 1902 (*3*); since then the interrenal and chromaffin tissues of about 80 species of teleosts have been described (*1*, *4*, *5*).

In order to extend these observations, over 125 species from 55 teleost families were collected from a variety of habitats (*6*). The anterior kidneys of these animals were fixed either in Bouin's or in Orth's (*7*) fluid. The latter fixative contains potassium dichromate, and was used to determine the distribution of chromaffin cells.

The interrenal morphology is extremely variable in these fishes. Furthermore, the surrounding tissues may be lymphoid or myeloid or both, and may or may not contain renal elements (*2*). The relationship of the interrenal to these tissues is also variable. Usually the interrenal is associated with the postcardinal veins or their branches or both, which ramify through the anterior kidney tissue. Chromaffin cells are always located within the walls of these veins. Often the interrenal and chromaffin tissues are separate, although more frequently there is some type of association between them.

When interrenal and chromaffin cells occur together around the veins within the anterior kidney, one of several possible arrangements may be found. The chromaffin tissue may be interspersed among the interrenal cells, as in the Cyprinidae and Cyprinodontidae (*5*). In the Gasterosteidae (*5*), clumps of interrenal alternate with clumps of chromaffin cells, the entire complex forming a cuff around the lumen of the postcardinal vein.

More often only the chromaffin cells are seen within the walls of these veins, either adjacent to the endothelium or embedded within the connective tissue of the vein wall. The interrenal is found external to both the vein wall and its associated chromaffin tissue. Such an arrangement is illustrated in Fig. 1 (*A*, *B*), which shows the interrenal and chromaffin tissue of a butterfly fish, *Chaetodon miliaris*. Even in the truly interspersed glands mentioned above, the chromaffin cells tend to be located closest to the vein lumen.

During the course of this investigation, an unusual arrangement of the interrenal and chromaffin tissues was seen. This arrangement has not been described previously, and has been observed only in fish from two closely related families: the Labridae (wrasses) and the Scaridae (parrot fishes). In some of these animals, the interrenal cells were found immediately adjacent to the endothelium of the veins, as a single layer of columnar cells. The chromaffin cells lie between the interrenal and the connective tissue of the vein wall (Fig. 1, *C*, *D*). This peculiar arrangement of cells was observed in all of the species of Scaridae examined, which included *Scarus dubius*, *S. formosus*, *S. perspicillatus*, and *Scarops rubroviolaceus*. In regions of the anterior kidney where the interrenal is markedly thickened, however, the interrenal and chromaffin cells were often interspersed.

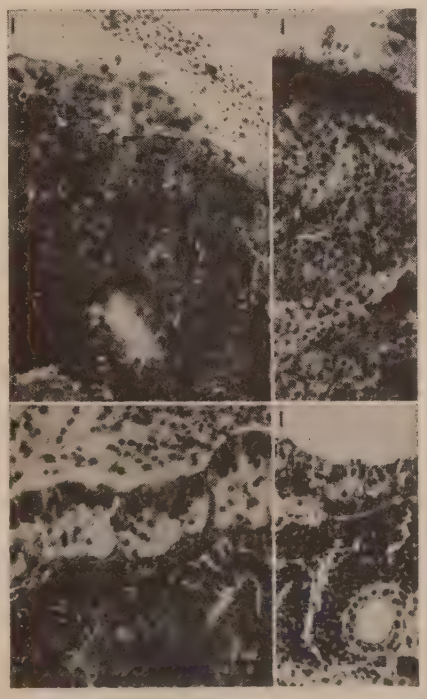

Fig. 1. Interrenal and chromaffin tissues associated with a branch of the postcardinal vein within the anterior kidney. Tissues were stained with hematoxylin and eosin; magnification is × 365. (*A*) *Chaetodon miliaris*, Bouin's fixation. (*B*) *Chaetodon miliaris*, Orth's fixation. (*C*) *Cheilinus rhodochrous*, Bouin's fixation. (*D*) *Cheilinus rhodochrous*, Orth's fixation. Abbreviations: *i*, interrenal cells; *c*, chromaffin cells (the positively reacting chromaffin cells appear dark after Orth's fixation); *l*, vein lumen.

Among the Labridae studied, the relationship between interrenal and chromaffin cells illustrated in Fig. 1 (*C*, *D*) was seen in the following species: *Cheilinus rhodochrous* (Fig. 1, *C*, *D*), *Cheilio inermis*, *Hemipteronotus baldwini*, *Novaculichthys taeniourus*, *N. woodi*, *Thalassoma duperreyi*, and *T. umbrostigma*. In most of these fishes, other arrangements were also seen, especially in those regions where the interrenal cells are stratified. In five other species of Labridae (*Bodianus bilunulatus*, *Coris flavovittata*, *C. ballieui*, *Cymolutes leclusei*, *Thalassoma ballieui*), this unusual relationship of interrenal and chromaffin cells was not observed.

Modifications of interrenal location and morphology may be related to the maintenance of an adequate blood supply to this gland, particularly as it increases in size. The function of the sinusoids visible in the interrenal of *Chaetodon* (Fig. 1*A*) may be performed by the larger blood vessel in *Cheilinus* (Fig. 1*C*), as long as only a single layer of interrenal cells lies adjacent to the vein lumen. It is noteworthy that the chromaffin cells maintain a relatively constant position in the vein wall despite marked variations in the distribution of interrenal tissue. The significance of these morphologic variations is not apparent, and attempts to correlate interrenal structure with habitat or taxonomic position of the species have thus far been unsuccessful (*8*).

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Two Shark Incidents at Eniwetok Atoll, Marshall Islands[1]

E. S. HOBSON,[2] F. MAUTIN,[3] and E. S. REESE[2]

THE TWO SHARK INCIDENTS described in this paper can be be considered as unprovoked shark attacks (Gilbert *et al.* 1960: 324). The importance of documenting these incidents is that they were observed by persons with some experience in the study of animal behavior. The great need of precise and detailed observational information on shark attacks has been stressed in recent reports on the subject (Coppleson, 1958: ix; Gilbert *et al.*, 1960: 323; Tester, 1960: 181). It is the belief of the authors that well-documented reports of shark incidents will contribute significantly to a clearer understanding of the stimulus situations in which shark attacks on man can be expected.

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FIRST INCIDENT

At noon on September 1, 1960, Hobson, Mautin, and Reese were engaged in spear fishing activities on a reef about 25 ft. below the surface and approximately 200 yd. from shore on the lagoon side of Parry Island, Eniwetok Atoll, Marshall Islands. Dr. Richard A. Boolootian of the University of California was in a 16-ft. skiff powered by a 35-hp. outboard motor which was idling nearby. The sea was calm, there was very little wind, and the sky was clear. Underwater visibility was in excess of 100 ft. Table 1 indicates the nature of the equipment that was being used. The spatial relationship of the divers to one another and to the skiff above the reef at the start and the end of the encounter is shown in Figure 1.

Hobson speared a 25-lb. grouper (*Plectropomus* sp.), which swam, trailing the spear, beneath a large coral boulder (rock 1, Fig. 1). Almost immediately three grey sharks (*Carcharhinus menisorrah*, Fig. 2), approximately 5–7 ft. in length, appeared from the deep water of the lagoon and began to circle the rock. Hobson observed (1) the sharks were swimming in an excited manner, and (2) they paid no attention to him floating quietly on the surface. Suddenly the grouper broke cover and dashed to a second large coral boulder (rock 2, Fig. 1). The three sharks immediately followed and began to circle the second rock.

Reese, who was directly above rock 2, noticed the sharks and was also impressed with the excited appearance of their swimming. Meanwhile, Hobson swam towards rock 2 to inform Mautin and Reese of the speared fish and the excited sharks in the area. At about this time Mautin observed the sharks and noted their excited state. All three began to swim toward the skiff, making a conscious effort not to produce an undue amount of commotion on the surface. There were now four or five sharks swimming in an excited manner on the reef below. Mautin, who was unarmed and farthest from the boat, was swimming strongly in an effort to join the others. Both Hobson and Reese observed that Mautin's swim-fins were breaking the surface of the water resulting in clouds of bubbles being carried beneath the surface at each down stroke.

The following sequence of events was very rapid. Almost simultaneously with the above observation, Mautin had reached a position almost above rock 2 (see Fig. 1). Suddenly one of the sharks rose from the bottom and swam very fast in a direct line towards Mautin. Mautin saw the approaching shark and rolled on his right side

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TABLE 1

PHYSICAL CHARACTERISTICS OF DIVERS AND NATURE OF EQUIPMENT USED AT TIME OF FIRST INCIDENT

	HOBSON[1]	MAUTIN	REESE
Weight	180	195	175
Height	5′ 10″	6′ 3″	5′ 9″
Skin color	brown	brown	brown
Bathing suit	khaki	none	gray & white
Swim fins	light blue	yellow	black
Mask	dark blue	white	dark blue
Snorkel	green	white	black
Spear	Hawaiian sling with 6-ft. free shaft	none	Hawaiian sling with 6-ft. free shaft

[1] Same equipment in second incident.

in order to face the onrushing shark. When the shark was very close he kicked it violently on the snout with his swim-fins. The shark veered away, circled once or twice behind Mautin and, upon the approach of Hobson and Reese, swam away. All three swimmers were now quiet in the water.

Immediately after the first shark left the bottom, a second shark began to swim toward the surface on the same course as the first shark. However, after approaching approximately halfway, it returned to the reef floor. It is suggested that the cessation of commotion on the surface

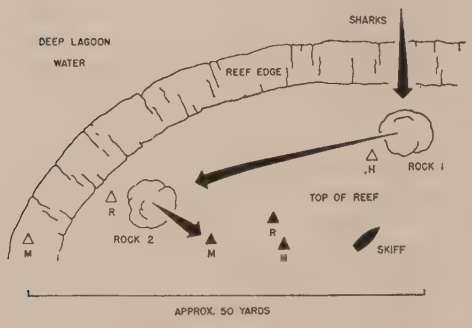

FIG. 1. Spatial relationship of divers to one another and to skiff above the coral reef at start of incident (white triangles) and at time of shark attack (black triangles). Letters beside triangles refer to diver which they represent, H = Hobson, M = Mautin, and R = Reese. Arrows indicate direction of movement of sharks.

and the presence of three swimmers resulted in the first shark swimming away and the second shark returning to the bottom.

Both Hobson and Reese were approximately 20–30 ft. from Mautin at the time the first shark attacked; however, there was no indication that the shark directed any attention toward them. It appeared that the shark was orienting to the agitation at the water's surface caused by Mautin's strong swimming. This observation supports the suggestion by Tester (1960: 183) and others that sharks are attracted by unusual commotion. A second possibility is that the shark was attracted to the bright yellow swim-fins which Mautin was wearing, but Hobson, on the basis of unpublished data, feels that this is unlikely.

Mautin gained the impression that the shark came for him believing he was the wounded fish, and that competition from the other sharks resulted in the direct and swift attack not prefaced by the usual cautious investigation.

The four to five sharks were still swimming in the same excited manner in the vicinity of rock 2 when the divers left the water.

SECOND INCIDENT

A second incident occurred the following day, September 2. Again the time was approximately noon, the sky was clear, and the surface of the lagoon was calm with underwater visibility in excess of 100 ft. Fish were being speared in shallow water along the beach at Sand Island adjacent to the deep water channel leading into the lagoon from the east. Hobson was in the water, and John C. Kay, a graduate student at the University of Hawaii, was standing off the beach in a 16-ft. skiff with the outboard motor idling. Hobson speared a 10-lb. parrot fish (Scaridae) which carried the spear shaft toward deeper water. About 35 yd. from the beach, at the edge of the reef, the bottom drops abruptly from approximately 15 ft. to over 100 ft. Grey sharks are common along this drop off. Hobson pursued the fish along the bottom, thus avoiding the surface commotion which had apparently attracted the attention of the sharks the day before. He overtook the injured fish on the bottom at a depth of approximately 15 ft. on the edge of the drop off. When the trailing spear

shaft was grasped, the fish struggled free. At the same instant a 6-ft. grey shark (*Carcharhinus menisorrah*) was upon Hobson, swimming at a slightly upward inclination directly at his face. The mouth of the shark was open, and movement of the jaws was distinctly recalled. Instinctively Hobson projected his right arm, catching the onrushing shark under the head. This, combined with a twisting, ducking motion, diverted the shark's forward rush over Hobson's left shoulder. The shark turned abruptly and circled so close that the spear which was still in the left hand could not be brought into play. As the shark circled, Hobson turned with it, pushing it away several times with his open hand until the shark was circling at a distance of approximately 4 ft.—enough room to bring the spear into use. The blunt end, which happened to be the end toward the shark, was used as a prod, and after a few jabs the shark opened its circle to approximately 10 ft. The shark appeared to be rapidly losing its aggressiveness. It seemed that apprehension toward the diver was now displacing the attack response which had asserted itself seconds before. As the boat approached, the shark fled into the adjacent deep water. The entire incident lasted only a few seconds and took place entirely on the bottom.

The shark was recognized by a deformation of the dorsal fin as one which had been involved in many of the behavior experiments conducted during the summer. It was, therefore, very familiar with the sight of humans in the water. There had been no apparent hesitation involved in its attack. Presumably its approach was made from deep water, coming over the edge of the reef which was within 10 ft. of the attack site. An approach from any other direction would have been observed.

Although the use of bare hands in warding off a shark has generally been discouraged (Gilbert *et al.*, 1960: 326), this was the only recourse in the present situation. The effectiveness of the hands in this case was no doubt largely due to the relatively small size of the shark. No lacerations of the hands occurred.

FIG. 2. *Carcharhinus menisorrah*, the species which was involved in both incidents, is common in the lagoons of most Pacific atolls, attaining a length of about 7 ft. (Photo: E. Hobson.)

TABLE 2

SUMMARY OF SIMILARITIES AND DIFFERENCES BETWEEN THE TWO INCIDENTS

	FIRST INCIDENT	SECOND INCIDENT
Activities at time of incident	spear fishing	spear fishing
Dead or injured fish present	yes: 1	yes: 1
Skin diving or SCUBA	skin diving	skin diving
Species of shark	*Carcharhinus menisorrah*	*Carcharhinus menisorrah*
Number of sharks in area	3–5	1
Size of sharks (length)	5–7 ft.	6 ft.
Date	Sep. 1, 1960	Sep. 2, 1960
Time of day	approx. noon	approx. noon
Condition of sky	clear, few clouds	clear, few clouds
Condition of sea	calm	calm
Location of incident	lagoon, Eniwetok	lagoon, Eniwetok
Distance from shore	200 yd.	35 yd.
Near deep water	yes	yes
Underwater visibility	100 ft. +	100 ft. +
Depth of water	25 ft.	15 ft.
Depth at which incident occurred	surface	15 ft., bottom
Water temperature	approx. 85° F.	approx. 85° F.
Nature of bottom	coral reef	coral reef
Number of persons in water	3	1
Nature of approach	direct, fast	direct, fast
Area of body approached	feet	head
Persistency of shark	moderate	considerable

DISCUSSION

The two incidents are summarized in Table 2. The authors believe that neither the coloration of the swimmer's equipment, the condition of the water, nor the time and nature of the day are significant. There are four major differences between the two incidents which are believed to be significant: (1) the number and relative positions of persons in the water, (2) the number of sharks in the vicinity, (3) the site of the incidents, the first on the surface, the second on the bottom, and (4) the location of the diver relative to the wounded fish. There are three major similarities between the two incidents which are believed to be significant: (1) both occurred in the vicinity of, and subsequent to, the spearing of a fish; (2) both occurred near deep water; and (3) both involved the same species of shark, which attacked without hesitation.

The last point may be explained by two facts. First, throughout the summer, Hobson, Mautin, and others noticed that *C. menisorrah* was a far more aggressive shark than the other two species which are common in the lagoon (*C. melanopterus* and *Triaenodon obesus*). This view is supported by Harry (1953: 48), who reports that the natives of the Tuamotus fear this species and claim it will attack man. Second, both incidents occurred within approximately a mile of where shark behavior experiments were conducted throughout the summer. Hobson recognized the shark in the second incident as having been a participant in these experiments, and it is probable that the sharks involved in the first incident had also had considerable experience with seeing human beings in the water. It is the belief of Hobson, Mautin, and others that the sharks became progressively bolder towards humans during the course of the summer. Thus it may be significant that both incidents occurred at the end of the summer.

It appears that one can generalize to this extent. Some species of sharks, for example *C. menisorrah,* are attracted to an area where there are injured fish. Apparently stimuli originating with the injured fish release an excited, highly motivated pattern of exploratory behavior in the shark. At such times many sharks seem to be particularly reactive to any unnatural disturbances created by humans in the general area.

This was illustrated by the response to the commotion at the surface of the water in the first incident. Furthermore, it seems they may also attack a human simply because he is located in an area of high concentration of the postulated stimulating factor, such as occurred in the second incident. In both incidents, visually directed thrusts at the sharks using hands, feet, or spear proved effective in momentarily warding off the attacking shark. Eibl-Eibesfeldt and Hass (1959: 746) and others have also noted the effectiveness of a pointed shaft as a shark deterrent.

CONCLUSIONS

1. Sharks may be expected to appear wherever spear-fishing activities are undertaken, particularly in those areas where aggressive species are known to occur.

2. When sharks appear under these circumstances they may attack a human located within the immediate area of the stimulating factor as well as humans associated with an unnatural amount of activity in the general area.

3. Sharks which have become familiar with the sight of humans in the water may be more likely to attack humans than those which have not.

4. The conclusion reached by others—that visually directed blows, preferably using a pointed instrument (such as a spear shaft), are effective in warding off sharks—is confirmed.

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